Severe *Streptococcus pyogenes* infections in Europe (Strep-EURO satellite symposium)

S1 Project Strep-EURO: a presentation

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Severe invasive group A streptococci (GAS; *Streptococcus pyogenes*) continue to evoke concern in many Western countries since high fatality rates and devastating complications are noted by streptococcal toxic shock syndrome, necrotizing fasciitis and others. The main objective of the project Strep-EURO is to improve understanding, at the European (EU) level, of the epidemiology of severe disease caused by GAS. The project is divided into seven work packages distributed between participating centers. We intend to improve systems for EU-wide surveillance by setting up protocols for identification of cases of severe GAS disease, collection of bacterial isolates and by construction of a database with clinical and laboratory information. A diagnostic network amongst EU and associated countries for characterization of GAS and rapid exchange of information will be created. To achieve an integrated picture of these infections, molecular methods for typing and clonal assessment of clinical isolates will be used. Identification methods for epidemiological markers will be harmonized at all participating laboratories. Strains will also be analyzed for antibiotic susceptibility, and resistance determinants will be traced. Emerging antibiotic resistance in GAS and its impact on treatment of severe disease will be studied. Furthermore, pathogenic aspects of severe GAS disease, such as proteolytic activation of the contact system, and selected virulence factors, such as surface proteins and superantigens, will be investigated. Finally, data obtained will be analyzed in order to allow for suggestions on new preventive measures against severe GAS disease.

S2 Molecular typing of group A streptococci

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Severe infections, common outbreaks, nonsuppurative complications and emerging antibiotic resistance are important reasons for epidemiological studies of group A streptococci (GAS). Classically, definite typing of GAS resides in identifiction of the M type using antiserum to the type-specific M protein; however, antisera for M typing are exceedingly difficult to raise and therefore seldom available even at specialized centers. Similarly, opacity factor (OF) typing GAS has only been used to a limited extent. As more commonly applied, GAS may be roughly classified into about 30 types by T-agglutination. New molecular methods for typing and clonal assessment of GAS include *emm*-typing by capture-PCR-ELISA, *emm*-gene N-terminal sequencing, *emm*-gene polymorhism, pulse-field gel electrophoresis (PFGE). For capture-PCR-ELISA, the part of the gene corresponding to the type-specific determinants of the M protein is amplified and then hybridized against gene probes of a number of M types, immobilized in a microtiter system. The captured DNA has been labeled with fluorescein and is detected by antibodies to fluorescein coupled with horse radish peroxidase. By PFGE, chromosomal DNA is cleaved by rare cutting endonucleases, such as *Sma*1, yielding approximately 15–30 very large DNA fragments. These pieces are then separated in a gel, the resulting pattern being used for typing and clonal identification of strains. For *emm*-gene polymorphism typing, PCR primers amplifying the major part of the *emm* gene are used. The amplicon is cleaved by two suitable restriction endonucleases and then analyzed by gel electrophoresis. By this method, even variation within a single M type can be diagnosed, which is of high importance for clonal identification. Recently, *emm*-gene N-terminal sequencing has been increasingly applied and resulted in the recognition of more than 150 *emm*-types. These correspond both to earlier known M-types and represent new types where serological identification has not been developed.

S3 Clonal identification of group A streptococci

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In the past, conventional typing based on the surface protein antigens T and M, sometimes supplemented by the serum opacity reaction (SOR) and anti-SOR typing, were relied on to study the epidemiology of *Streptococcus pyogenes* ('the group A streptococcus') infections. However, many questions related to the spread and outbreaks of severe streptococcal disease could not be answered when using these traditional methods. In the strep-EURO project, molecular methods will be applied to elucidate questions such as the following:

1 Are *S. pyogenes* strains found in recent severe infections different from 'historical' isolates of the same M (*emm*) type;
2 are there predominant 'invasive' clones which can be differentiated from 'noninvasive' isolates (e.g. from pharyngitis);
3 does a change occur in predominant clones over the study time;
4 what is the rate of geographic variation among invasive isolates;
5 can the spread of antibiotic resistance in *S. pyogenes* be explained using molecular typing and subtyping methods;
6 which different subtyping methods have to be employed to answer these and other questions in the epidemiology of severe *S. pyogenes* disease?

It is planned to use the following methods on the streptococcal strains collected in the project:

1 On all isolates: *emm*-typing (see presentation of Dr A. Jasir);
2 on selected strains: multilocus sequence typing (MLST), pulsed field gel electrophoresis (PFGE), sequencing of the genes *sic* and *drs* (for appropriate *emm*-types); sequencing of other genes e.g. of superantigens and other virulence factors known to display genetic polymorphism.

The value of these and other molecular subtyping methods will be established in the course of the project.

S4 Invasive disease caused by group A streptococci in Sweden during 1997 and 2002

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Group A Streptococci (GAS) may cause a variety of symptoms ranging from superficial skin infections and tonsillitis to severe invasive disease including necrotizing fasciitis and streptococcal toxic shock syndrome. In this study, we performed a prospective surveillance of invasive infections caused by GAS in Sweden during 1997 and 2002. Clinical isolates were collected from patients with invasive disease (years 1997 and 2002) and also from noninvasive cases (year 1997) in Sweden. Isolates were characterized by antibiotic susceptibility testing, T-typing, and by using molecular methods such as PFGE, *emm*-sequencing, MLST, and superantigen determination. Also, clinical and epidemiological data were collected about the patients, and were correlated to clonality.

S5 High frequency of group A streptococci type M 89 invasive infections in Italy

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During 1994–96, 80 Group A Streptococci (GAS) isolates from cases of invasive infections were received for serotyping. As high number of strains (32/80, 40%) were nontypable (NT) with our standard M-typing antisera, the *emm* genes were amplified by PCR and sequenced. Forty-four percent of NT strains belonged to *emm*89 type, which resulted, together with type M1 (16/80 cases), the type most frequently (19/80 cases) responsible for invasive infections in Italy. In particular, type *emm*89 was significantly associated with necrotizing fasciitis (8/26 cases) and bacteremia (8/27 cases). One strain was isolated from a case of meningitis and 2/24 strains were from streptococcal toxic shock syndrome. No case associated with *emm*89 was fatal. All *emm*89-type strains were positive for the production of opacity factor (OF) and had a T-pattern corresponding to B3264, 3/B3264, or 3/13/B3264. All strains were high producers of protease; all strains carried speB, whereas were negative for *speC* and *speA* genes. The pulsed-field gel electrophoresis profile of all *emm*89 strains was similar but distinct from those strains belonging to other types or untypable. Strains belonging to *emm*89 were also isolated from several cases of skin lesions and GAS pharyngitis. It looks likely that pharyngeal strains represent a reservoir for invasisve diseasese.

S6 Emergence of a clone of *S. pyogenes* resistant to bacitracin

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Objectives: A prospective survey of acute pharyngitis was conducted from November 2000 to June 2001, in Bourgogne, a region of France, involving 20 general practioners. The aim was to study the frequency of antimicrobial resistance of *Streptococcus pyogenes* responsible for pharyngitis.
Methods: Streptococcal pharyngitis was first diagnosed by the positivity of the Biostar Strep A Optical Immunoassay (International Microbio). It is a rapid test that detects the group A antigen. A second throat swab was cultured on Columbia blood agar plate and allowed to isolate 247 strains of *S. pyogenes* from 282 patients. Biotyping, T-typing, and *emm*-typing were carried out with the Rapid ID Strep 32 (BioMerieux), agglutination method, and sequence analysis, respectively. Pulsed-field gel electrophoresis was performed on strains with similar markers. Susceptibility to 12 different antibiotics, including bacitracin, was tested by the disk diffusion method. The minimal inhibitory concentrations (MICs) of macrolides (erythromycin, azithromycin, and josamycin), clindamycin, and tetracyclin were determined by the agar dilution method according to the guidelines of the 'Comité de l'Antibiogramme de la Société Française de Microbiologie'(CA-SFM). The major genetic determinants of macrolides resistance (ermB, ermTR, and mefA) were investigated by multiplex PCR assay.
Results: Thirty out of 247 isolates of *S. pyogenes* (12.1%) were found to be resistant to bacitracin. All these strains were also resistant to high levels of macrolides and clindamycin (MICs $\geq$ 32 mg/L) associated with the *ermB* gene. High levels of resistance to kanamycin and streptomycin were detected in 30 and 27 strains, respectively. Most of these multiresistant strains were of biotype 1, serotype T28, and type *emm*28, and represented more than 40% of the *emm*28 strains. The pulsed-field gel electrophoresis showed a homogeneity among these strains.
Conclusion: These results confirm the spread of a strain of *S. pyogenes* biotype 1, T28, *emm*28, characterized by an unusual resistance to bacitracin. The spread of this clone during an 8-month period among the whole area of the survey might have been favored by the association of high levels of resistance to macrolides. Bacitracin-resistant strains have already been isolated from invasive infections. Therefore, the detection of *S. pyogenes* should not be relied by the positivity of the bacitracin susceptibility test.

S7 Antibiotic resistance of group A streptococci in Romania during 1998–2002

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Group A streptococci (GAS) strains collected by the National Reference Laboratory for streptococci during 1998–2002 were isolated mainly from respiratory infections (acute tonsillitis and scarlet fever), skin infections, and healthy carriers. T-agglutination subtyping was performed and T pattern 5/11/12/27 was predominant. GAS strains included in this study were tested for susceptibility to 10 antibiotics (penicillin, cefotaxime, cefuroxime, cephalothin, erythromycin, clindamycin, tetracycline, chloramphenicol, ofloxacin, and vancomycin). Our results revealed that isolates from Romania were uniformly susceptible to β-lactams, but to a variable degree resistant to alternative antibiotics, such as macrolides, lincosamides, tetracycline, and fluoroquinolones. The frequency of tetracycline resistance within GAS was 51%, whereas erythromycin resistance was rather rare accounting for only 6.5%. Phenotypes of erythromycin resistance were identified further by a double disk diffusion test. M phenotype was the most common. On the basis of a triple disk diffusion test, strains resistant to macrolide, lincosamide, and streptogramin (MLS) were assigned further to the corresponding inducible MLS subtypes. In Romania, the current resistance rate of GAS to erythromycin seems to remain relatively low compared with those of other European countries, but continued surveillance of macrolide resistance in GAS is recommended.

S8 Antibiotic resistance among group A streptococci

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An overview of the most important problems of resistance in GAS will be presented based on the literature and our own experience from Denmark. The major antibiotic resistance problems in GAS during the last two decades have been resistance toward the macrolides and tetracycline. Incidence of erythromycin resistance levels of up to 100% in some third-world countries has been reported, while levels of up to 50% have been published from European countries. In Denmark, the incidence is still low, i.e. in the range of 0–5%. In most studies, a close relationship between macrolide use and development of resistance has been proven. On the other hand, erythromycin resistance has remained low in Denmark in spite of a rather high macrolide use. The distribution of macrolide resistance genes is different in European countries, the reason for which is unknown. The genotypes can easily be determined from the phenotypic characteristics, e.g. from susceptibility testing with erythromycin and clindamycin. Tetracyklin resistance levels in Danish GAS has been surprisingly high considering that these drugs are not used for treatment of GAS infections. The genetic background recently elucidated will be presented. Penicillin resistance in GAS has never been reported, but there is still discussion whether penicillin tolerance exists in these bacteria. A few reports on PBP changes in GAS have been published. Whether this could precede real resistance towards penicillins should be subjected to future investigations.

Designing successful antibiotic strategies (Symposium arranged with the BSAC)

S42 Systematic review of interventions to improve antibiotic prescribing: hospital inpatients

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Although 80–90% of all antibiotic prescriptions for human use are issued in the community, resistance to these drugs is a more serious and acute problem in the hospital setting. In response to the inexorable increase in resistance rates, government and other authoritative bodies have called for reductions in inappropriate antibiotic prescribing, with the expectation that the trend will be reversed. However, none of these bodies has specified the processes by which this outcome is to be achieved and, to date, there have been no published guidelines, based on systematic reviews, which have defined the optimal interventions or combination of interventions. In 1993, the British Society for Antimicrobial Chemotherapy convened a working party with the specific aim of addressing this issue. In the course of performing a systematic/Cochrane review, the working party produced a comprehensive database comprising of 665 articles published since 1980. Only 296 of these contained original data and all but 77 (26%) of the 296 papers were excluded on the grounds of inadequate methodology, i.e. they were not randomized control trials, controlled before and after studies, or interrupted time series with adequate numbers of data points before and after the intervention. The final 77 articles are currently being evaluated according to a protocol devised by the Cochrane Effective Practice and Organization of Care Group. However, despite fulfilling the criteria for inclusion, many of these articles suffer from one or more flaws in their design and/or execution. It is likely, therefore, that at least some of the recommendations made by the working party will be based on expert consensus, rather than robust evidence. For the time being, it is reasonable to assume that a combination of interventions will be necessary in order to have a maximum impact on the prescribing practices. Potential measures that might comprise an antibiotic control plan include a formulary, with restrictions on the prescribing of selected drugs, some form of constraint on the duration of prescriptions, evidence-based guidelines for antibiotic usage, laboratory control, and educational measures.

Re-evaluating pharmacokinetics/pharmacodynamics relationships: a critical appraisal

S43 The relationships between bacterial killing, the MIC, PK/PD indices and antibacterial effect

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The MIC is the in vitro reference value to describe the activity of an antibiotic against microorganisms. This value is obtained by incubation of a defined inoculum over a certain period of time, and thus does not represent the effect of an antimicrobial at a specific point in time, but rather the effect of the antibiotic over a period of time, the incubation period. The MIC is read at a fixed concentration. In vivo however, concentrations decline over time and the effect of the antibiotic thus changes over time. At some concentration, the killing effect of the antibiotic is less than a certain value and bacteria will start growing. Using the Hill equation with variable slope, it is shown that this concentration is not equal to the MIC. Simulations using ranges of values for killing rate, growth rate and slope factor show that for beta-lactam antibiotics the static concentration is close to the MIC value and that this may explain why concentrations in vivo need to be above the MIC, while regrowth of bacteria occurs when concentrations decline below the MIC. For concentration dependent antibiotics such as aminoglycosides and quinolones, the static concentration is shown not to be equal to the MIC, and in general is much lower. This observation may explain why bacteria do not regrow immediately after concentrations have declined below the MIC and thus, the presence of phenomena such as Post Antibiotic Effect.

S44 The use of in vitro models to predict emergence of resistance

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Pharmacokinetic/pharmacodynamic (pK/pD) in vitro models have been increasingly used to assess dosing regimens in terms of reducing the risk of resistance occuring as well as predictions on efficacy. In vitro models have an advantage over animal systems in that much larger bacterial populations (up to 10E + 10 cfu) can be exposed to antibiotic. Resistance can be measured by changes in MIC before or after drug exposure, the ability of the bacteria to grow on agar containing a single MIC multiple (i.e. 4× MIC) or on agar containing a range of MIC multiples (i.e. 1× MIC, 2× MIC, 4× MIC, etc.). If this latter approach is used, then changes in the bacterial population profile can be assessed. Most work in this area has been conducted on the fluoroquinolone class, and the following factors are important in: (a) predicting resistance in bacterial species, (b) drug exposure measured by AUC/MIC or Cmax/MIC, (c) duration of exposure, (d) agent tested, (e) initial bacterial population heterogeneity, and perhaps, (f) existing resistance mechanism. The relationship between drug exposure and resistance follows an inverse quadratic relationship, being lowest at low and high AUC/MIC values. The mutant prevention concentration (MPC) may also have a role in prediction of resistance which is not inconsistent with the concept of a quadratic relationship outlined above. *Staphylococcus aureus* and *P. aeruginosa* are commonly used in model systems as resistance occurs more rapidly than other species such as *S. pneumoniae*. It remains to be established if extrapolations made on these organisms can be justified for others.

S46 How predictive is PK/PD for antimicrobial agents

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MIC of antimicrobials, in combination with plasma concentrations achieved during therapy, are generally used to predict clinical and microbiological efficacy. Based on in vitro data, β-lactams and macrolides are said to be time-dependent and concentration-independent, whereas aminosides and fluoroquinolones are concentration-dependent and time-independent. Time-kill, dynamic testing, and animal models have been used to establish predictors of efficacy such as time >MIC, AUC/MIC, and Cmax/MIC. For aminosides and fluoroquinolones, it is thought that Cmax/MIC ratios must exceed 10, or AUC/MIC ratios must be 125 (for Gram-negative organisms) or 30 (for Gram-positive organisms). Yet, the relationship between PK/PD parameters and clinical outcome has rarely been investigated in man. Moreover, existing clinical trials suffer from several drawbacks such as their retrospective nature. PK/PD parameters are usually calculated on the basis of MIC and AUC tables rather than on individually determined values. The predictability of these parameters should now be revised. Optimization of Cmax/MIC ratio maximizes β-lactam efficacy in infections caused by penicillin nonsusceptible pneumococci, while $t >$ MIC and T_{max} or AUC/MIC are the best dynamic predictors of the microbiologic and clinical efficacy of fluoroquinolones in patients with pneumococcal infection. AUC breakpoints such as 125 cannot be adopted for antibiotics of all classes because very different serum concentration profiles can result in the same AUC. Moreover, there are considerable variations in PK/PD calculations in individuals, and predictors of efficacy appear to be drug- and pathogen-specific. In the case of mild community-acquired infections, antibiotics should ideally be administered at relatively high dosage and short dosing intervals. In contrast, in difficult situations (e.g. immunodeficiency or high MIC values), individually adjusted PK/PD parameters might be a valid basis for the choice of dosage schedule. One of the recent strategies for fighting antibiotic resistance is that few mutants will arise if antibiotic concentrations block the most resistant mutant. This led to the concept of the mutant prevention concentration (MPC), which prevents the growth of first-step mutants. Antibiotics and dosage schedules should now be assessed by taking into account the time that drug concentrations in sera and tissue exceed the MPC.

Improving diagnosis and treatment of viral infections of the central nervous system (Joint symposium arranged with the IDSA)

S47 Diagnosis of herpesvirus infections of the CNS: a virologist's challenge

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Human herpesviruses, in particular, herpes simplex virus types 1 and 2, and also cytomegalovirus, Epstein-Barr virus, varicella-zoster virus, and human herpesvirus 6 and 7 are responsible for numerous infections of the central nervous system (CNS). Due to the large spectrum of clinical signs and symtoms associated with herpesvirus infections of the CNS, many of which are nonspecific or atypical, diagnosis often represents a challenge for both clinicians and microbiologists. A presumptive diagnosis is based on the patient's history, clinical signs, EEG profiles, radiological imaging, and CSF analyses, even though these investigations may be normal at early stages of disease. The availability of CSF PCR-based diagnostic techniques has allowed for rapid and accurate diagnosis, leading to specific therapeutic interventions and shortened patient stays. CSF–PCR provides a higher sensitivity and specificity than viral isolation and serological tests, such as intrathecal antibodies. The use of CSF–PCR has expanded, for instance, the awareness of mild or atypical HSV encephalitis that represents 16–25% of the cases. Besides CSF–PCR, PCR analysis of CNS tissue also plays an important role in the diagnosis of herpetic infections of the CNS. However, due to the capability of the herpesviruses to persist in the host tissue in a latent state, PCR results obtained from tissue specimens must be interpreted cautiously, since viral genomic material may be present even in the absence of an active viral infection. Negative PCR results provide evidence against a role of herpesviruses as the causative agents. In addition, analysis of specific viral gene transcripts in selected CNS cell preparations by RT-PCR techniques can provide a definitive insight as to whether the herpesvirus is passively carried by

bystander inflammatory cells, is latent, or is in an active replication state. In addition to qualitative PCR, quantitative PCR, that allows the number of viral DNA copies in the CSF to be measured, has shown significant prognostic value. Finally, newly developed quantitative real-time PCR assays, making results available at a much earlier time, significantly enhance the impact of the diagnosis on clinical intervention, reducing the need for empiric treatment, and providing the opportunity to monitor the ongoing therapy from the virological viewpoint.

Urinary tract infections: new insights into virulence and pathogenesis (Joint symposium arranged by ESCMID/ICAAC)

S51 Virulence in *Klebsiella*: use of molecular techniques and animal models

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Background: *Klebsiella pneumoniae* is a common cause of urinary tract infection (UTI) and pneumonia, especially in immunocompromised individuals. Epidemiological studies have revealed that *K. pneumoniae* infections are frequently preceded by colonization of the intestine. The gastrointestinal tract is host to a very complex microflora, which is the most important reservoir for transmission of bacteria.
Objectives: To identify genes involved in the ability of *K. pneumoniae* to colonize the intestine and infect the urinary tract.
Methods: A novel multiscreening signature-tagged mutagenesis assay (MS-STM) was developed. In the MS-STM assay, PCR-amplified tags present in the inoculum as well as recovered pools from each infection model were simultaneously subjected to hybridization using each specific tag as a probe. Hereby, screenings of a mutant library in more than one infection model was significantly eased compared to the traditional STM methodology.
Results and conclusions: Mutants were identified being attenuated in both the intestinal colonization as well as the UTI model, and more important mutants attenuated in the UTI model only were identified. Transposon insertion sites in attenuated mutants were, among others, in genes encoding well-known *K. pneumoniae* virulence factors such as lipopolysaccharide and capsule, as well as genes of so far unknown function. Additional studies have shown the importance of *K. pneumoniae* capsule in UTI. The bacteria seem to have specific strategies for colonization and survival in the different host organs. The regulation of presentation/expression of these features is still unknown.

S53 Bacterial virulence factors: are they important to know for the treating physician?

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Urinary tract infection (UTI) is the result of interactions between bacterial virulence and host defense mechanisms at several levels. In individuals with normal urinary tracts, to cause infection, bacteria have to be equipped with certain properties, i.e. virulence factors, making it possible to overcome host defenses. Such infections are designated as uncomplicated, and typically occur in young, healthy, nonpregnant females. The dominating uropathogen, *Escherichia coli*, possesses an array of virulence properties that participate at different stages of the infectious process. Adhesins mediating attachment to epithelial cells seem to play an important role in colonization of mucosal surfaces and the ascent of bacteria into the urinary tract. *E. coli* isolated in urine from women with uncomplicated pyelonephritis more often belong to certain O : K : H serotypes and more frequently express virulence factors like P fimbriae, hemolysin, and aerobactin than *E. coli* strains in the fecal flora of persons not having UTI or those isolated from patients with asymptomatic bacteriuria or acute cystitis. However, when host defenses are compromised, as in patients with functional or structural abnormalities of the urinary tract, infections are often caused by a diversity of less virulent *E. coli* strains. Obvious clear-cut correlations with specified types of UTIs are missing. One reason for this could be that many virulence factors seem to be encoded by flexible genetic elements called pathogenicity islands and that the regulation of gene expression may act independently or in concert. In all, the pathogenetic role of adhesins and other virulence factors such as toxins and siderophores have not been fully elucidated. As an example, only a small number of *E. coli* strains from men with various types of UTI have been characterized as regards virulence properties. In a retrospective study, *E. coli* isolates from 74 men with febrile UTI were phenotypically analyzed. A wide array of O : K : H serotypes commonly associated with acute pyelonephritis in women were identified. There was a higher frequency of hemolytic strains among patients with febrile UTI (74%) and a lower frequency of P fimbriated (51%) and aerobactin-positive strains (46%) than previously encountered in women with uncomplicated acute pyelonephritis. A prospective study investigating both phenotype and genotype corroborates the findings. Are these results important to know for the treating physician?

Health care workers as a source of infection (Joint symposium arranged with SHEA)

S55 Prevention of transmission of HBV and HCV from health care workers to patients: towards a European consensus?

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Recently, representatives from several countries met to devise common recommendations for prevention of transmission of viral hepatitis from medical personnel to patients. In the past, health care workers (HCWs) were voluntarily vaccinated against hepatitis B to protect them from occupational infection. Over 45 reports describe transmission of hepatitis B virus (HBV) from HCWs to patients. HCWs who are immune for hepatitis B cannot acquire and transmit HBV to patients during exposure-prone procedures (EPPs). Obviously, patients should be protected from iatrogenic HBV infection by mandatory vaccination of HCWs. Can vaccination be mandatory? It seems justified that an employer demands vaccination of applicants for a job involving EPPs. However, HCWs already employed may refuse vaccination on various grounds. It seems reasonable that refusing HCWs undergo mandatory screening for HBV infection regularly. When vaccinated, HCWs show an antibody response below 100 IU/L. The presence of HBV must be ruled out because a significant number of HBV carriers have low levels of anti-HBs. Recently, a surgeon who showed 252 IU/L anti-HBs after vaccination was found to be HBV infected. He transmitted HBV to three patients. This incident suggests that all EPP-performing HCWs should be tested for presence of HBV, irrespective of the vaccination status. In Europe, the proportion of HBV carriers among HCWs ranges from 0.3 to 3%. The US excludes HBV-positive HCWs from performing EPPs if they test positive for the HBV e-antigen. In addition, the UK and Ireland exclude e-antigen negative HCWs with HBV viral loads above 1000 cp/mL. In Holland, HBV-positive HCWs are excluded from EPPs if the viral load is above 100.000 cp/mL. The consensus panel proposes a cut-off level of 10.000 cp/mL, balancing the risk of transmission and the loss of medical specialists. Since 1992, the WHO advocates worldwide vaccination of infants against HBV. Several

north-west European countries decided to limit vaccination to at-risk groups. As a result of this policy, the general population remained vulnerable to HBV infection, and in the future patients, HCWs and blood donors still may acquire and transmit HBV. The estimated prevalence of hepatitis C virus (HCV) infection among European HCWs ranges from 0.2 to 3%. The consensus panel did not reach agreement on how to manage HCV infected HCWs who perform EPPs.

S57 Health care workers as a source for hospital-acquired diarrhea

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Objective: Discuss the evidence implicating health care workers (HCW) as a source of spread of organisms causing diarrhea in the hospital, particularly *Clostridium difficile*.
Methods: Literature review, personal experience, and unpublished study data.
Results: *C. difficile*-associated diarrhea (CDAD) is the most frequently identified cause of nosocomial diarrhea. This organism persistently contaminates the hospital environment through the formation of spores that persist for prolonged periods. The hands of hospital workers have been documented to be contaminated frequently by *C. difficile* following contact with patients who are asymptomatically colonized or who have CDAD, or by contact with the environment of these patients. HCW do not carry *C. difficile* in their stools more frequently than nonhealth care workers, and are at risk of CDAD only if they are taking antibiotics. Evidence that healthcare workers transmit *C. difficile* are indirect, and based on a significant decrease in CDAD rates in a controlled trial of glove wearing by HCW ($P < 0.05$). Cases of CDAD caused by a specific strain of *C. difficile* have been associated with care by the same HCW teams on a hospital ward and were not associated with geographic proximity of the patients. Waterless alcohol hand hygiene products are not sporicidal and may not be effective in removing *C. difficile* spores, but their use has not been documented to be associated with increased CDAD rates to date.
Conclusion: HCW are a source of *C. difficile* transmission to patients, presumably via hand contact. Gloving, and possibly hand washing, have demonstrated benefit in reducing CDAD rates.

S58 Prevention and control of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant pathogens

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Objectives: To discuss control of antimicrobial-resistant pathogens in healthcare settings.
Methods: Review of national surveillance data and published intervention studies.
Results: Since 1980, Centers for Disease Control and Prevention (CDC) National Nosocomial Infections Surveillance (NNIS) system data have shown that the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) keeps increasing every year; currently approximately 50% of *S. aureus* isolates causing hospital-acquired infections are resistant to methicillin. Similarly, since 1989, vancomycin-resistant enterococci (VRE) have rapidly emerged; currently, approximately 25% of enterococci causing hospital-acquired infections are resistant to vancomycin. Since 1997, eight patients have been diagnosed with vancomycin-intermediate resistant *S. aureus* (VISA) infections and, in 2002, the first vancomycin-resistant *S. aureus* (VRSA) infection was documented in USA. Over the past decade, approximately 150 000 patients have died of these infections in US hospitals. Antibiotic use (emergence) and patient-to-patient transmission (spread) are the two most important risk factors for infections caused by antibiotic-resistant pathogens. CDC guidelines recommend that patients with 'epidemiologically important antibiotic-resistant pathogens', like MRSA, VRE, VISA, or VRSA, should be cared for using contact precautions. A variety of studies have shown that person-to-person transmission is facilitated by the unknown iceberg of the colonized patient. Recently, several studies have used active surveillance cultures to detect both infected and colonized patients; these studies have shown that MRSA can be controlled in a facility, and that VRE was eradicated or significantly reduced in 32 healthcare facilities in a region.
Conclusion: If all healthcare facility personnel start implementing programs of active surveillance cultures for these pathogens, healthcare-associated MRSA and VRE infection rates (and the resulting emergence of community-acquired MRSA, and hospital-acquired VISA and VRSA) could begin falling (or fail to further emerge) for the first time in decades.

Insight into the epidemiology of bacterial resistance

O59 Referred cases of urinary tract infections caused by strains of *Escherichia coli* producing extended-spectrum beta-lactamases in a pediatric hospital

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Objective: To conduct an epidemiologic survey of urinary tract infections (UTIs) caused by *Escherichia coli* strains producing extended spectrum beta-lactamases (EC-ESBLs), occurring in infants referred to our hospital, without obvious predisposing factors.
Methods: During routine infection – control monitoring of multiresistant pathogens, a cluster of EC-ESBL was noted among urine pathogens isolated from infants in December 1998. Worksheets were reviewed retrospectively, and the first such isolate was noted to have occurred in December 1998. Subsequently, it was decided to collect epidemiologic data for all EC-ESBL urine isolates retrospectively from December 1998 to January 1998 and prospectively from January 1999 to December 1999. All cases of infection were classified as referred to or acquired in the hospital. Susceptibility to antimicrobials was tested by a disk diffusion method according to the NCCLS criteria. Production of ESBLs was detected by the double disk synergy test (DDST). Molecular typing was carried out by pulse – field gel electrophoresis (PFGE).
Results: During the study period, 40 episodes of UTI caused by EC-ESBL were diagnosed in 40 patients. Of these, only four were considered to be acquired in the hospital. The remaining 36 cases were classified as 'referred'. The study population consisted of 28 boys and 12 girls. Of these, 33 (82%) patients were younger than 12 months. All these infants, except three, had their first episode of UTI. Urinary tract abnormalities were found in 40% of patients, with vesicoureteric reflux being predominant. EC-ESBL strains accounted for 3.1 and 6.5% of total *E. coli* isolated from urine cultures during 1998 and 1999, respectively. All EC-ESBL isolates were susceptible to imipenem and ciprofloxacin. Resistance rates to other antibiotics were, as follows: cefepime, 2.5%; amoxicillin/clavulanate and piperacillin/tazobactam, 5%; gentamicin, 85%; amikacin, netilmicin and tobramycin, 87.5%; trimethoprim-sulfamethoxazole, 20%. A single phenotype (A) was observed among infants (20/33, 60%) from December 1998 to December 1999. All these infants had been born from November 1998 to March 1999 in the same maternity hospital (MH-I) in Athens. PFGE revealed nine distinct types (A-I). Twenty of 28 tested isolates belonged to PFGE type A; all had originated from MH-I.
Conclusions: None of the referred infants had obvious predisposing factors associated with infection caused by ESBL strains. The majority of EC-ESBL strains were found to be acquired in MHs.

O60 Enterococci and coagulase-negative staphylococci disseminate frequently between intensive care unit patients

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The intensive care unit (ICU) is often proposed to be the part of the hospital with the highest frequency of nosocomial infections. Enterococci and coagulase-negative staphylococci (CNS) both constitute increasingly important nosocomial problems worldwide, especially in severely ill and immunocompromised patients, mainly due to intrinsic and acquired antimicrobial resistance

Objectives: To investigate the colonization pattern in the respiratory tract of mechanically ventilated ICU patients and bacterial transmission within and between patients, with specific regard to enterococci and CNS.
Methods: Twenty consecutive patients undergoing incubation at an ICU in a Swedish hospital were included. Samples were collected from the oropharynx, the subglottic space, the stomach and the trachea within 24 h of incubation and then every third day until day 18, thereafter every fifth day until day 33. Enterococca and CNS isolates ($n = 170$ and 199, respectively) were genotyped with pulsed-field gel electrophoresis. The minimum inhibitory concentration (MIC) was determined for five clinical relevant antimicrobial agents by the agar dilution method.
Results: Enterococci – Seven clones occurred each in at least two patients and 13 of 17 colonized patients were involved in at least one transmission event. One of the patients shared strains with six other patients. CNS – Seven clones were each isolated from two or more patients. Eleven out of 20 patients were involved in between one, and up to six, transmission events. The risk of acquiring an epidemic strain increased with prolonged treatment at the ICU. The majority of the strains expressed multiple resistance, but none of the enterococci was vancomycin resistant.
Conclusions: Transmission of resistant enterococci and CNS between mechanically ventilated patients seems to be frequent since a total of 17/20 patients were involved in at least one transmission event. Although transmission of bacteria does not necessarily lead to infection, it is nevertheless an indication that infection control measures can be improved. Registration of nosocomial infection rates will greatly underestimate the true number of bacterial transmissions between hospitalized patients.

O61 Incidence of enterococcal surface protein in glycopeptide-resistant *Enterococcus faecium* during its emergence in a Greek intensive care unit

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Objectives: Enterococcal surface protein (*esp*) gene, a potent virulent factor, has been recently detected in glycopeptide-resistant *E. faecium* (GREF) infection-derived isolates spreading in hospitals (*Lancet* 2001;357:853-55). We searched for the *esp* gene in GREF strains during their emergence in the ICU of a tertiary care hospital.
Methods: Between February 1999, when first detected, and April 2001, 21 GREF strains of VanA phenotype were isolated from the same number of patients at the multidisciplinary ICU of 'Evangelismos' Hospital. Clinical data from all these patients were collected. Identification of strains and antibiotic susceptibility testing by MIC were performed. All isolates were examined for the presence of *vanA* and *vanB* genes by polymerase chain reaction (PCR) analysis. Also, the presence of the *esp* gene was detected by esp PCR.
Results: All patients were acutely ill, aged 51 + 18 years, with a high Apache II score on admission (19 + 5), long length of ICU stay (50 days, median, range 12–320) and had a high ICU mortality (48%). GREF acquisition was noted late during ICU stay (30 days, median, range 9–150, postadmission) following a long duration of vancomycin and/or third-generation cephalosporin therapy. The vanA glycopeptide resistance genotype was found in all isolates. The *esp* gene was present in 17 out of the 21 (90%) tested isolates. Blood was the most common source of the *esp* gene positive strains ($n = 7$), followed by drainage ($n = 4$), pus ($n = 3$), intravascular catheters ($n = 2$) and urine ($n = 1$). The four *esp* gene negative strains were detected in fluid of wound or drainage. No significant association was detected between ICU mortality and the presence of the *esp* gene.
Conclusion: A high incidence of *esp* gene was detected in infection-derived *E. faecium* strains, in our ICU, especially in blood isolates. This finding documents the emergence and dissemination of highly virulent *E. faecium* strains among ICU patients.

O62 Antibiotic susceptibility among bacteria in 29 Swedish intensive care units

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Objectives: To evaluate the incidence of antibiotic susceptibility among bacteria isolated from patients in 29 Swedish ICUs during 1999–2000.
Methods: Consecutive specimens collected on clinical indications from ICU patients, were cultured and tested for susceptibility to routinely used antibiotics. Susceptibility testing was performed on 7766 initial clinical isolates using a disk diffusion method according to the Swedish Reference Group for Antibiotics (SRGA).
Results: The most dominating isolates were in percentage: CoNS 17.8, followed by *Candida* spp. 13.1, *Staphylococcus aureus* 8.4, *Escherichia coli* 8.2%, *Enterococcus faecalis* 6.9, *Streptococcus* spp. 6.8, *Klebsiella* spp. 4.4, *Pseudomonas aeruginosa* 3.9, *Enterobacter* spp. 3.5, *Enterococcus faecium* 3.5. Ceftazidime/Cefotaxime showed a broad activity (>96%) against *E. coli* and *Klebsiella* spp. but also against *P. aeruginosa* (ceftazidime), *Serratia* spp. and *S. maltophilia* (88–92%), but lower rates for *Enterobacter* spp. (67%) and *Acinetobacter* spp. (20%). High susceptibility to ciprofloxacin was seen among *E. coli*, *Enterobacter* spp., *Klebsiella* spp. and *Serratia* spp. (mean 90–95%). Corresponding figures were for; *Acinetobacter* spp. 89%, *P. aeruginosa* 85%, *S. maltophilia* 68%, *S. aureus* 15%, CoNS 3.6%, *E. faecalis* 0.8% and *E. faecium* 0.4%. All species showed high susceptibility to imipenem (96%) except *P. aeruginosa* (70%), *E. faecium* (11%) and *S. maltophilia* (0%). Also for Netilmicin a high susceptibility was found among *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *P. aeruginosa*, *S. aureus*, *Serratia* spp. (>96%) and *Acinetobacter* spp. (91%) but not for CoNS (54%). The MRSA-rate was only 2% and most MRSA-isolates came from one ICU. The oxacillin resistance among CoNS was >70%. The local susceptibility rate among *P. aeruginosa* ranged 57–100% for imipenem, 82–100% for ceftazidime and 67–100% for ciprofloxacin.
Conclusions: The results in this study were in line with previous Swedish and Scandinavian ICU surveillance studies showing high antibiotic susceptibility among most bacteria except for lower rates of antibiotic susceptibility among *P. aeruginosa*. There was no correlation between local consumption of carbapenem, ceftazidime and ciprofloxacin and resistance to these drugs in *P. aeruginosa* ($P = 0.87$, 0.22 and 0.87, respectively) which indicated a need for local hygienic interventions against the transmission of resistant *P. aeruginosa* rather than restrictions of antibiotic usage.

O63 Epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in a Tel Aviv medical centre

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Objectives: Multi-drug resistant (MDR) *Pseudomonas aeruginosa* (PA) may result in adverse outcomes. This study was designed to understand the spread and to determine risk factors (RF) for MDR PA at our institution.
Methods: Molecular analysis using PFGE of selected MDR PA, collected during a 10 month period. Epidemiological investigation and a case-control study comparing cases with control patients that were matched to cases by ward, calendar time, and length of stay (LOS) until inclusion. Data on demographics, primary diagnosis, active comorbidities (AC), procedures/devices, and treatment were collected from patients' records. Analyses were performed using conditional logistic regression.
Results: During the study period 82 cases with MDR PA from 20 wards were identified. Thirty-six unique patients isolates were typed using PFGE and revealed 12 different clones. Two of the clones dominated; clone A (10 cases) which caused an outbreak in the ICU (7 cases within 3 months) and spread to other wards, and clone B (9 cases) which did not cluster in time and/or space. In the case-control study 82 cases (age 65, 60% male, 1.2 AC), were included and had mean LOS to isolation of 17 days. Cases were compared to 82 matched controls (age 63, 50% male, 1.3 AC). In univariate analysis: transfer from another institution, previous ICU stay, invasive device, number of antimicrobials, and severity of illness, were significant RF. AC of malignancy was protective. The multivariate model included ICU stay (OR 10.1, $P = 0.04$), being bedridden (OR 3.5, $P = 0.04$), having invasive devices (OR 13.9, $P = 0.02$), number of antibiotics (OR 1.8, $P = 0.01$) and malignancy (OR 0.2, $P = 0.03$). When each class of antibiotics was included in the model third generation cephalosporins (OR 9.6, $P = 0.003$) and aminoglycosides (OR 6.1, $P = 0.04$) were unique RF.
Conclusions: MDR PA belonged to multiple clones and appeared on many wards. A dominant clone spread from ICU to other wards. RF analysis identified previous ICU stay, being bedridden, and multiple invasive devices were significant RF for MDR PA. Heavy antibiotic treatment, and particularly treatment with third generation cephalosporins and aminoglycosides are important RF for MDR PA.

O64 First results of *Escherichia coli* resistance monitored by the European Antimicrobial Resistance Surveillance System (EARSS)

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Objectives: In 2001, routinely generated antimicrobial susceptibility test results for invasive *E. coli* were added to the European Antimicrobial Resistance Surveillance System (EARSS). We present the first susceptibility results for aminopenicillins (amoxicillin/ampicillin), aminoglycosides (gentamicin/tobramycin), fluoroquinolones (ciprofloxacin/ofloxacin) and third-generation cephalosporins (ceftazidime and cefotaxime/ceftriaxone).
Methods: The EARSS protocol requires laboratories to report interpretative susceptibility results (S, I, R) of *E. coli* isolates. Only first isolates of a patient are accepted. Between January 2001 and October 2002, EARSS received results of 20 511 isolates from 332 laboratories in 22 countries. We calculated proportions of resistant (R) *E. coli* isolates per country. Median resistance levels were those reported by the median country after ranking countries from low to high resistance.
Results: Aminopenicillin resistance in *E. coli* was above 25% in all countries (median: 44%). Resistance to gentamicin and tobramycin exceeded 10% in some countries but the median was only 4%. Countries with the highest resistance levels to aminopenicillins (southern and eastern Europe) also exhibited the highest resistance rates to aminoglycosides. Fluoroquinolone resistance appeared to be a problem in many countries. The median resistance levels were 8% in 2001 and 12% in 2002, which is noticeably higher than the levels reported in previous publications. Resistance to third generation cephalosporins was still uncommon, although levels were higher than 5% in many eastern European countries. Resistance to three or more different classes of antimicrobials occurred in 7.5% of *E. coli* strains.
Conclusion: In *E. coli*, resistance to several classes of antimicrobials is common throughout Europe, mostly in southern and eastern countries and in Israel. The high resistance levels to single antimicrobial classes, the relatively high prevalence of multidrug resistance and the high level of fluoroquinolone resistance are of concern and will be closely monitored.

O65 Susceptibility patterns are unchanged over three years in community-acquired lower respiratory *Streptococcus pneumoniae* and *Haemophilus influenzae* in the UK and Ireland

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Objective: To monitor trends over time in the antimicrobial susceptibility of pathogens associated with community-acquired lower respiratory tract infection.
Methods: Two thousand and twenty-seven *S. pneumoniae* and 2810 *H. influenzae* from lower respiratory specimens were collected from 20 laboratories in the UK and Ireland in the winters of 1999–2000, 2000–01 and 2001–02, excluding duplicates within 2 weeks and patients in hospital more than 48 h. Isolates were centrally tested by BSAC agar dilution MIC method and categorized by BSAC breakpoints.
Results: The tables show the percentage of resistant [intermediate] isolates.

S. pneumoniae antimicrobial $R \geq$ [I range] (mg/L)	1999–2000 $n = 661$	2000–2001 $n = 667$	2001–2002 $n = 699$
Penicillin, 2 [0.12-1]	0.9 [9.8]	0.3 [9.9]	0.7 [7.0]
Amoxicillin, 2	1.2	1.3	0.9
Cefaclor, 2	12.0	10.5	7.6
Cefuroxime, 2	8.2	6.3	5.4
Cefotaxime, 2	0.2	0.1	0.0
Erythromycin, 1	13.3	11.2	12.2
Clindamycin, 1	5.9	4.3	4.3
Ciprofloxacin, 4[≤2]	5.4 [94.6]	5.1 [94.9]	8.7 [91.3]
Tetracycline, 2	8.9	7.9	5.9
Trimethoprim, 1	100.0	100.0	100.0
Moxifloxacin, 2	0.3	0.3	1.1

H. influenzae antimicrobial $R \geq$ [I range] (mg/L)	1999–2000 $n = 936$	2000–2001 $n = 958$	2001–2002 $n = 916$
Ampicillin, 2	15.2	16.0	16.3
Amoxicillin, 2	21.2	20.8	22.1
Amoxicillin-clavulanate, 2	7.8	4.0	5.2
Cefaclor, 2	98.8	92.8	91.3
Cefuroxime, 2	19.0	15.8	17.2
Cefotaxime, 2	0.0	0.0	0.0
Erythromycin, 16 [1-8]	3.7 [95.7]	2.8 [96.1]	4.8 [94.4]
Ciprofloxacin, 2	0.1	0.0	0.1
Tetracycline, 2	3.5	2.6	2.4
Trimethoprim, 1	9.7	11.4	17.0
Moxifloxacin, 2	0.0	0.0	0.1

The results, like those of other databases, point to a small reduction in resistance of *S. pneumoniae* to beta-lactams. Hints of a possible upward trend in resistance can be seen only for trimethoprim/*H. influenzae* and ciprofloxacin/*S. pneumoniae*, the latter probably due to the breakpoint falling on the shoulder of the distribution. The full MIC distributions show that susceptibility patterns have generally remained very similar throughout the three study years.
Conclusion: In contrast to reports of increasing resistance from some other European countries, patterns of resistance in community-acquired lower respiratory tract pathogens in the UK and Ireland appear to have been stable over the last three years.

O66 Susceptibility to penicillin of invasive compared with respiratory *Streptococcus pneumoniae* and relationship with age

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Objective: To compare antimicrobial susceptibility in invasive and community-acquired lower respiratory tract *S. pneumoniae*, taking account of patient age.
Methods: One thousand three hundred and twenty-eight *S pneumoniae* from lower respiratory specimens were collected from 20 laboratories in the UK and Ireland in the winters of 1999–2000 and 2000–2001, excluding duplicates within 2 weeks and patients in hospital more than 48 h. Two hundred and twenty-seven *S pneumoniae* from blood cultures were collected from 24 laboratories in 2001. Isolates were tested centrally by BSAC agar dilution MIC method (respiratory isolates at GR Micro and bacteremia at ARMRL), and categorized by BSAC breakpoints (mg/L).
Results: We have shown previously that respiratory *S. pneumoniae* show a U-shaped relationship between patient age and nonsusceptibility to penicillin and other antimicrobials, significant in multivariate analysis. The bacteremia isolates showed the same relationship, and there was no evidence of any difference in susceptibility between isolates from blood and lower respiratory sources. The table shows the results for penicillin.

	Bacteremia		Respiratory	
Age range	Number R+I/total	%R+I	Number R+I/total	%R+I
0-4	2/18	11	13/79	16
5–24	0/12	0	8/71	11
25–49	1/39	3	16/254	6
50–74	6/75	8	59/622	9
75+	11/81	14	43/301	14
Age not known	0/2		1/1	
Overall	20/227	10	140/1328	11

R + I = resistant + intermediate, i.e. MIC ≥ 0.12 mg/L.

Amoxicillin, cefotaxime, ciprofloxacin, erythromycin and tetracycline were also tested against both respiratory and blood isolates. There was no difference in susceptibility between the two, except with ciprofloxacin where the blood isolates typically had MICs one doubling dilution higher than respiratory giving an apparent but probably spurious difference in resistance of 31 vs. 5%.
Conclusions: Antimicrobial susceptibility in *S. pneumoniae* depends on patient age, but there is little evidence of difference between invasive and noninvasive disease in the UK and Ireland.

O67 Presence of class 1 integrons in Enterobacteriaceae from human, food, and animal reservoirs

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Introduction: Class 1 integrons are associated with multiresistance (MR) in Enterobacteriaceae due to their frequent presence on resistance determinant carrying conjugative plasmids. The role of different reservoirs in the transmission of resistance determinants is poorly understood. In previous studies we observed 11 different integron types (I–XI) at the neurodivision of our hospital [AAC 2001, 45 : 2961] and 19% of the isolates from newly admitted patients possessed an integron, including types V and VII [JCM. 2002, 40 : 3038]. The aim was to characterize integrons in Enterobacteriaceae from human, food and animal samples.
Methods: Gentamicin- and cotrimoxazole-resistant clinical isolates were obtained from dogs ($n = 14$) and horses ($n = 27$). *Escherichia coli* screening isolates were obtained from swine ($n = 620$), calves ($n = 310$) and chickens ($n = 621$). Screening isolates were tested for nine noncross-reacting antibiotics. Meat samples were obtained at two supermarkets. All isolates were characterized by integrase (int)- and CS-specific PCR. CS-PCR amplification products were characterized by restriction enzyme analysis.
Results: Among the isolates from dogs 10 were int-positive and at least two types of integron were found, including type V and XII. Among the 20 int-positive horse isolates at least four integrons were discovered, including types V, VIII, and XII. A total of 21 isolates (3.3%) from swine were MR. Twelve isolates were int-positive and at least two integron types could be identified, including type VII. Forty-five isolates (14.5%) from calves were MR and 35 were int-positive. At least six different integron types were present, including types V, VII and XII. Fifty chicken isolates (8.1%) were MR, 38 (6.1%) were int-positive and at least four integron types were found, including types V, VII, and XII. Both meat samples were int-positive, but only one sample was also CS-PCR positive. In this sample integron types V and VII were present. These data suggest that, integrons from farm animals contribute in part to the antibiotic resistance observed in the hospital setting. It can be argued that isolates harboring an integron are more prone to acquire additional resistance determinants.
Conclusion: Several types of integron are shared among human, food and animal isolates indicating that resistance determinants present among (farm) animals may contribute to the problem of MR Enterobacteriaceae in the hospital setting.

O68 Quality assessment of antibiotic susceptibility testing by laboratories participating in the European Antimicrobial Resistance Surveillance System (EARSS) in 2002

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Objectives: The goal of this third external quality assurance (EQA) exercise of EARSS is the ongoing assessment of comparability in susceptibility test results across countries and guidelines.
Methods: A set of five strains; *S. aureus* U2A1556 (mecA, penA, rpoB, ant4′), *E. faecium* U2A805 (vanB, aph2′-aac6′, ant3′9, erm), *E. coli* U2A1557 (IRT), *E. coli* U2A1526 (blaCTX-M, aac3-V, ant3′, gyrA), and *S. pneumoniae* (penR, aph3′-III, ant3′9, ermB, cat, parC, TpRSuR), were provided by CRAB (Centre National de Référence des Antibiotiques) and distributed by UK-NEQAS (United Kingdom National External Quality Assessment Scheme) to the laboratories participating in EARSS. The laboratories were asked to report methods and guidelines used for speciation, MIC-determination (when performed) and clinical susceptibility categorization (S, I and R). Results were considered 'concordant' if the reported categorization agreed with the designated interpretation of three reference laboratories.
Results: Overall, 642 (93%) of 690 laboratories from 26 countries reported results. Most of them used NCCLS guidelines (71%). For the *E. faecium*, the overall concordance was high for amoxicillin/ampicillin (98%), gentamicin (98%), vancomycin (90%) and teicoplanin (96%) but low for species identification (87%). For the *S. pneumoniae* the overall concordance was high for oxacillin (98%), penicillinG (98%), erythromycin (95%) and clindamycin (93%) but low for ceftriaxone/cefotaxime (48%). Both *E. coli* strains had a high concordance for amoxicillin/ampicillin (98–100%), gentamicin (99%) and ciprofloxacin (96–100%). Importantly, the concordance for detection of ESBL production was high (93–95%). The results for the *S. aureus* are not available yet.
Conclusion: The third EQA exercise of EARSS had again a high overall concordance, confirming that the resistance surveillance as monitored by EARSS is valid. However, the 13% misidentification of the *E. faecium* should be dealt with by the laboratories. The low concordance for the susceptibility of the *S. pneumoniae* to ceftriaxone/cefotaxime most probably occurred because the low breakpoint ($S \leq 0.5$ mg/L, NCCLS) was only one dilution step from the intended result (MIC = 1 mg/L).

Emerging bacterial problems in pediatric infections

O69 Longitudinal carriage of Pneumococcus in kindergarten children in Hong Kong

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Introduction: Young children under the age of five are recognized as a risk group for carriage of antimicrobial resistant *Streptococcus pneumoniae* and are more susceptible to pneumococcal infections. A cross sectional study of kindergarten children in Hong Kong had shown a carriage rate of 19% with more than 50% of strains being nonsusceptible to penicillin. However, the pattern of prolonged carriage of penicillin resistant strains in this community is unknown.
Objectives: To investigate longitudinal nasopharyngeal carriage of *S. pneumoniae* among children attending kindergarten. The antimicrobial resistance patterns and risk factors for persistence were also investigated.
Methods: Nasopharyngeal swabs were collected from 680 children attending six different kindergartens on three occasions over a 5 month period and questionnaires providing demographics and medical history for risk factors were completed by their parents. *S. pneumoniae* was isolated and antibiotic susceptibilities determined using standard methods.
Results: On the first occasion, the carriage of *S. pneumoniae* was 19.7% (134) whereas the carriage rate on the second occasion decreased to 12.3% (75) and to 10.2% (70) on the third occasion. Thirty-three (24.6%) children of the 134 initially carrying *S. pneumoniae* were found to be carriers on the first two samplings. Of these 33 isolates, 63.6% were not susceptible to penicillin. On the third sampling only eight children of the original carriers were still colonized with *S. pneumoniae* and all strains were penicillin resistant. Seventy-five percent of persistently carried strains were multiply drug resistant. On each occasion other children had acquired *S. pneumoniae* and 10 children who had been noncolonized on the first sampling were positive on the second and third occasions. Age and antibiotic prescription within the last month were found to be risk factors for resistance. Sex, family size, number of siblings, physician consultation and hospitalization were not significantly associated with carriage.
Conclusion: This is the first study of longitudinal carriage of *S. pneumoniae* performed in Hong Kong. Results for carriage are similar to those observed elsewhere in that the majority of isolates were only carried for a short period of time. However, rates of antibiotic resistance are much higher than those in most developed countries. Persistent carriage was found to be more common for antibiotic resistant strains.

O70 Invasive pneumococci in the pediatric population of Germany: Epidemiology and coverage of the 7 valent pneumococcal conjugate vaccine, 1997–2001

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Objectives: Pneumococcal disease is a major cause of morbidity and mortality, especially among children. A nation-wide study of invasive pneumococcal disease among children <16 years of age was started in 1997 in Germany.
Methods: Pediatric hospitals and their clinical microbiological laboratories were asked to report invasive pneumococcal infections among children and to

send the pneumococcal isolates to the NRCS for confirmation of species diagnosis, antibiotic susceptibility testing by the microbroth dilution method and serotyping by the quellung reaction. Multilocus sequence typing (MLST) was performed according to standard methods.
Results: Two thousand two hundred and eight cases were reported by the laboratories and hospitals. Capsular typing was carried out for 892 isolates and showed serotypes 14 (24.1%), 1 (8%), 18C (7.4%), 19F (7.4%), 23F (7.1%), and 6B (5.9%) to be the most prevalent. The percentage of cases per age group covered by the 7-valent pneumococcal conjugate vaccine including the potentially cross-protective serotype six A was 69.5% (age group, 6–11 months), 76.8% (1 years) and 74.6% (2–4 years). Three hundred and fifty isolates of pneumococcal meningitis were serotyped and the coverage of the 7-valent vaccine among children of 1 year of age was 81.2%. Erythromycin A resistance has increased from 12.9% in 1997 to 27.4% in 2001, which is mostly due to the increase of the incidence of serotype 14 from 16.3% in 1997 to 28.1% in 2001 (almost 50% of strains of serotype 14 are erythromycin-resistant). The percentage of penicillin resistance (I + R) was 5.6%. four (0.4%) isolates were highly penicillin-resistant (MIC > 2 mg/L). Fifty-eight penicillin resistant (I + R) strains were characterized by serotyping and MLST. Serotypes 23F (22.4%) and 14 (20.7%) were the most prevalent serotypes among penicillin resistant isolates. 34.5% of all sequence types of the 58 penicillin resistant isolates were Spanish clones with variable sequence types mainly ST-557 (8.6%) and ST-156 (8.6%).
Conclusions: Macrolide resistance is increasing in Germany. Penicillin and cefotaxime resistance rates still are one of the lowest world-wide. The 7-valent conjugate vaccine covers over 90% of all antibiotic resistant pneumococcal strains.

O71 Changing epidemiology of purulent Meningitis in children due to HIV infection

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Background: The impact that HIV infection has had on the epidemiology of purulent meningitis in children has not been well characterized.
Objective: To determine the etiology and clinical differences in purulent meningitis in HIV infected (HIV-positive) and HIV uninfected (HIV-negative) children.
Methods: Children with purulent meningitis were enrolled between March 2001 and March 2002 and their hospital records reviewed.
Results: One hundred and forty-two of the 154 children had a definitive HIV result, of which 44% were HIV-positive. The median age of HIV-positive and HIV-negative children was 8.5 months and 50% were male. The proportion of organisms isolated between HIV-positive and HIV-negative children, respectively, ($P < 0.0001$) were *S. pneumoniae* 75 vs. 26%: *Hemophilus influenzae* type b 11 vs. 41%: *Neisseria meningitidis* 5 vs. 16%, *S. agalacticiae* 5 vs. 10% In addition there was one case of *Cryptococcus neoformans* and *Escherichia coli* and no organism was identified in 2 and 5% of HIV-positive and HIV-negative children, respectively. Difference on cerebro-spinal fluid (CSF) analysis between HIV-positive and HIV-negative children included a lower neutrophil (median 249 vs. 400 cells/mL, $P = 0.02$ and lymphocyte count (median 25 vs. 70 cells/mL, $P = 0.02$) Gram stain (97 vs. 80%, $P = 0.002$) and culture (97 vs. 70% cells/mL $P = 0.002$) on CSF were more likely to be positive in HIV-positive than in HIV-negative children, respectively. Neurologic morbidity (31 vs. 29%) and mortality (28 vs. 16%) did not differ between HIV-positive and HIV-negative children, respectively. In HIV-positive children, meningitis due to *S. pneumoniae* was associated with a higher mortality than organisms (34 vs. 7%, respectively, $P = 0.004$).
Conclusion: The HIV epidemic has resulted in *S. pneumoniae* replacing *H. influenzae* type b as the dominant cause in children. An attenuated immune response to bacteria invading into CSF may explain differences noted in CSF analysis between HIV-positive and HIV-negative children.

O72 Impact of wide vaccination of children with pneumococcal conjugate vaccines on antibiotic resistance

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Paris, F

Objectives: Strains of *Streptococcus pneumoniae* resistant to penicillins have widely spread in the community in the last decades. New conjugate vaccines, which protect against both invasive disease and asymptomatic carriage, may help control this evolution. Here, we investigate the impact of the introduction of a conjugate pneumococcal vaccine for a given proportion of children on carriage of *S. pneumoniae* and of resistant strains in particular.
Methods: We have developed a mathematical model of the carriage of *S. pneumoniae* in an age-structured population. Bacterial strains are described by their serotype and a resistance level which is susceptible to increase with exposure to antibiotics; they spread in the community through interindividual transmission. A 7-valent vaccine is administered to a portion of the children under 1 year-old. The vaccine protects against carriage of the serotypes included in its composition. The parameters describing the natural history of colonization of *S. pneumoniae*, the frequency of contacts and the characteristics of antibiotic treatments were estimated from the literature. We investigated the changes in the proportion of carriers and in the distribution of resistance levels in terms of penicillin G MIC, both with and without vaccination.
Results: With vaccination, carriage of vaccine-type strains decreases to very low levels, typically in 20–30 years in epidemiologically realistic conditions. Simultaneously, strains not included in the vaccine spread in the community, so that the overall carriage rate remains stable. Surprisingly, vaccination does not affect the extent at which antibiotic resistance is selected. In all cases, the distribution of resistance levels peaks on high levels (MIC > 2 mg/L) after 20 years. The same results are obtained even with a vaccine optimally designed to include all serotypes currently exhibiting a decreased susceptibility to penicillin G.
Conclusion: Serotype replacement phenomenon rapidly follows vaccination, so that the overall carriage rate is not subject to major changes. Moreover, the distribution of resistance levels of all pneumococcal strains is the same after 20 years, irrespective of vaccination. This mathematical modeling suggests that vaccination alone may not be successful in controlling the burden of antibiotic resistance in *S. pneumoniae*.

O73 Impact of pneumococcal conjugate vaccines and antibiotic policies on the incidence of meningitis due to penicillin G-resistant *S. pneumoniae*

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Paris, F

Objectives: *S. pneumoniae* is among the leading causes of bacterial meningitis in the western world. Moreover, the outcome of the disease may worsen now that strains of *S. pneumoniae* with high levels of resistance to penicillin G are increasingly common. New conjugate vaccines, which protect against both invasive disease and asymptomatic carriage, may help decrease the burden of resistance. However, their long-term effects on disease and on resistance selection are not well known yet. Using mathematical modeling, we investigated the impact of a conjugate pneumococcal vaccine on the incidence of *S. pneumoniae* meningitis and among these infections, the portion of cases resistant to penicillin G.
Methods: We have developed a mathematical model of the selection of pneumococcal resistance to penicillin in an age-structured population. Zero to 100% of children under 1 year-old receive a conjugate vaccine which protects them against carriage of seven serotypes included in its formulation but not against other serotypes. The model takes into account the main parameters describing the natural history of colonization of *S. pneumoniae*, the frequency of contacts and the characteristics of antibiotic treatments, which were estimated from the literature. We investigated the time changes of the incidence rate of *S. pneumoniae* meningitis and in particular of penicillin G resistant cases. We also studied the impact of different treatment policies intended to reduce antibiotic exposure of children on the number of cases prevented by vaccination over 15 years.
Results: With vaccination, the carriage rate of *S. pneumoniae* – all strains combined – remains stable. In the same respect, at most 5% of the overall number of meningitis cases in 15 years can be prevented by vaccination. After approximately 20 years, penicillin G resistant meningitis constitute the near totality of cases of *S. pneumoniae* meningitis, independently of vaccination. However, vaccination combined to a strategy where the treatment frequency of all children is reduced by half enables the prevention of up to 25% of resistant meningitis cases in a 15 year period. This gain remains large even for vaccination rates as low as 20%.
Conclusion: Vaccination alone may not be sufficient to decrease the burden of resistant pneumococcal meningitis. However, reducing antibiotic use after the

introduction of vaccination would reduce the incidence of highly resistant cases and therefore improve outcomes in this disease.

O74 Impact of routine Hib vaccination in the Czech Republic and analysis of *Hemophilus influenzae* isolates by molecular methods

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Objectives: The aim of the study was to assess the impact of routine Hib vaccination and to study the clonality of *Hemophilus influenzae* b (Hib) isolated in an active surveillance program.
Methods: Routine vaccination of infants against invasive disease caused by Hib under 1 year of age was introduced in the Czech Republic in July 2001. The active surveillance of invasive Hib disease was introduced in the Czech Republic nation-wide in January 1999. *H. influenzae* isolated in the active surveillance program in 2001 were investigated using classical methods (serotyping, biotyping) and 20 selected Hib isolates by molecular methods (polymerase chain reaction – PCR, randomly ampified polymorphic DNA analysis – RAPD, pulsed-field gel electrophoresis – PFGE and multilocus sequence typing – MLST).
Results: A total incidence of Hib invasive disease ranged between 1.0 and 1.1/1 00 000 population before the introduction of routine Hib vaccination (in 1999 and 2000). The highest incidences in both years were in the age groups 0–11 months (17.1/1 00 000, and 15.6/1 00 000, respectively) and 1–4 years (17.4/1 00 000, and 20.9/1 00 000, respectively). The active surveillance data for 2001 and 2002 indicate the decrease of Hib invasive disease in the target age group (0–11 months) after the introduction of routine Hib vaccination, in which the age specific morbidity was 15.6/1 00 000 in 2001 and 3.3/1 00 000 in 2002). A total number of 249 isolates was obtained in the active surveillance program in 2001. Among those strains 184 were *H. influenzae* and 64 of these were Hib isolated from invasive disease. Biotyping of 63 Hib strains from invasive disease showed biotype I. Twenty Hib strains isolated in first quarter of 2001 were typed by molecular methods. Eighteen of them showed identical RAPD patterns and 18 identical PFGE profiles with minor differences. Six isolates investigated by MLST showed ST-6.
Conclusion: The results of active surveillance indicate decrease of Hib invasive disease in the target age group under the influence of mass Hib vaccination of infants. Hib strains isolated from invasive disease showed homogeneity in RAPD, PFGE and MLST patterns.
Acknowledgements: This study was supported by research grant NI/6803-3 of the Internal Grant Agency of Ministry of Health of the Czech Republic and made use of the Multi Locus Sequence Typing website (http://haemophilus.mlst.net). We thank Dr K. Jolley (University of Oxford, UK) for kind editing of the text.

O75 Clonal analysis of *Neisseria meningitidis* and vaccination strategy in the Czech Republic

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Objectives: Clonal analysis of meningococcal populations allows to assess the epidemiological situation and to recommend appropriate vaccination strategy for the country.
Methods: Strains isolated from invasive meningococcal disease (IMD) are sent to the National Reference Laboratory for Meningococcal Infections in Prague for further investigation by classical methods (slide agglutination, Whole Cell ELISA) and molecular methods (multilocus electrophoresis – MLEE and multilocus sequence typing – MLST). MLST is made according to the method described at the MLST website (http://neisseria.mlst.net). The results of strains isolated from IMD in the period 1980–2001 are presented.
Results: An emergency epidemiological situation of IMD started in the Czech Republic in 1993, when a new clone, *N. meningitidis* C, ET-15/37, ST-11, occurred. The new meningococcal clone caused increased IMD morbidity and case fatality rate. After 7 years of high prevalence of that clone, we have noticed the prevalence of serogroup B since 2000. However, the strains of serogroup B belong to the hypervirulent complexes (ET-5/ST-32 complex and ST-18 complex) and case fatality rate remains at a high level. A strategy of targeted vaccination of parts of the population at highest risk was adopted in 1993. This targeted vaccination has been used less frequently now, as we have noticed a decreasing incidence of IMD and a decreasing percentage of serogroup C. Mass vaccination with meningococcal conjugate C vaccine is not planned.
Conclusion: The emergency situation caused by hypervirulent complex *N. meningitidis* C, ET-15/37, ST-11 is over in the Czech Republic, without mass vaccination, and hypervirulent complexes of *N. meningitidis* B, ST-32 and ST-18, have started to replace serogroup C strains even though they do not reach such a high percentage as *N. meningitidis* C, ST-11 in the past. Mass vaccination with meningococcal conjugate C vaccine is not planned in this epidemiological situation.
Acknowledgements: This study was supported by research grants NI/6882-3 and NI/7109-3 of the Internal Grant Agency of Ministry of Health of the Czech Republic and EU project no. QLK2-CT-2001-01436 and made use of the Multi Locus Sequence Typing website (http://neisseria.mlst.net) sited at the University of Oxford and funded by the Wellcome Trust. We thank Dr K. Jolley (University of Oxford, UK) for kind editing of the text.

O76 Early intestinal bacterial colonization and necrotizing enterocolitis in premature infants: The putative role of *Clostridium*

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Necrotizing enterocolitis (NEC) is among the most severe conditions that can affects preterm infants. Although the etiology of NEC remains unknown, initial bacterial colonization could play a pivotal role in the development of NEC. To further explore the putative relationship between pathogen microorganisms and NEC, we conducted a prospective case-control study in 12 preterm infants with a new approach based on molecular techniques. Over an inclusion period of 24 months, 12 neonates of <34 weeks admitted in the neonatal unit were enrolled in a prospective study aimed at a description of the pattern of bacterial colonization in that population of infants. Within this study group, three infants happened to develop cataclysmic NEC and subsequently died. The group therefore includes three cases of NEC, and nine control infants without evidence of NEC, who were matched for gestational age and birth weight. Stools samples were saved at weekly intervals from all infants. Polymerase chain reaction and temporal temperature gradient gel electrophoresis of 16S ribosomal DNA were used to detect the establishment of bacterial communities in the digestive tract. A salient feature of the bacteriological pattern was observed only in the three infants who later developed NEC: a band corresponding to the *Clostridium perfringens* subgroup could be detected in early samples, and prior to diagnosis, whereas there was no evidence for this specific band in any of the nine controls. To our knowledge, the current report is first to demonstrate that the use of molecular techniques based on the study of bacterial 16S rRNA genes allowed the recognition of a *Clostridium perfringens* species in the first 2 weeks of life of three infants who later displayed symptoms of NEC. A significant temporal relationship was thus established between early colonization by *Clostridium* and the later development of NEC. Compared with conventional bacteriological culturing methods, the use of this new molecular approach to analyze the gastrointestinal ecosystem should therefore allow a more complete and rapid assessment of intestinal flora. The current data do not, however, constitute definite proof that the identified bacterial species was a causative agent in the development of NEC. But they outline the promise of this new technique based on molecular biology, and suggest that large scale studies on a much wider population at high risk of NEC may be warranted.

O77 Bacteremia due to *Pseudomonas* spp. other than *Pseudomonas aeruginosa* in children

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Objectives: To assess risk factors and outcome of bacteremia due to *Pseudomonas* spp. other than *Pseudomonas aeruginosa* in children.
Mehods: Among 240 cases of bacteremia within 3 years nationwide survey of Pseudomonas bacteremia, 11 (4.8%) were due to Non-aeruginosa *Pseudomonas* spp. and the rest due to *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia*. Here we present 11 cases of bacteremia in

immunocompromised children caused by *Pseudomonas* spp. other than *Pseudomonas aeruginosa*. We compared *Pseudomonas* bacteremias with X2 tst (EPI INFO computerized package). Blood cultures were incubated within the BACTEC (BBL) system and identified with VITEK centralized system (Bio Mérieux), antimicrobial susceptibilities were tested with disk diffusion test according to the NCCLS.

Results: All patients with bacteremia survived, one case did not even receive therapy: a heart transplant child, which was discharged from the hospital day before the positive blood culture was reported (after 5 days of incubation). All 10 children were successfully cured with ceftazidime (4), cefotaxim (4), cefepime (1) and azthreonam (1).

Conclusions: Surprisingly, the mortality in our group of non-aeruginosa *Pseudomonas* spp. bacteremia patients was different than we have seen in child with *Pseudomonas aeruginosa* bacteremia (20–33%) and Acinetobacter baumannii bacteremia (26%). We have no explanation for this difference. Also antimicrobial susceptibility of *Pseudomonas putida* and other *Pseudomonas* spp. was higher in comparing to *Pseudomonas aeruginosa*, where among 169 bacteremias, 32% were resistant to ciprofloxacin and 7–10% to ceftazidime.

O78 Enteroaggregative *Escherichia coli* associated with acute diarrhea in children, a case-control study

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Objectives: Enteroaggregative *Escherichia coli* (EAEC) is an emerging foodborne pathogen in developed as well as developing countries. In the present case-control study the role of EAEC in acute diarrhea among children under 5 years of age was investigated in Karaj, Tehran province, Iran. PCR of mixed culture, which has been evaluated as a highly sensitive method, was used for detection of EAEC in fecal samples.

Methods: During a 3 month period form May to July of 2002, 102 acute diarrhea patients, under 5 years of age, from different medical centers in the city of Karaj and 29 matched controls without diarrhea were included in the study. DNA was extracted from the primary mixed culture of fecal samples and subjected to PCR by a pair of primers targeting 630 base pair region of pCVD432 plasmid. As many colonies as required, were assayed for finding the isolate carrying the target sequence. The chi-square test was used for statistical analysis.

Results: Among the 102 acute diarrhea patients under 5 years of age investigated in our study (Median: 10 months, range: 2 months to 5 years, Interquartile range: 6–18 months), 37 (36.3%) were positive for EAEC, while only three (10.3%) of controls yielded EAEC in their stool specimens. The characteristics and the clinical symptoms of the patients with acute diarrhea were vomiting in 31 (69.6%), watery stool in 46 (45.5%), mucus in stool in 59 (42.2%), bloody stool in seven (6.9%), and fever in four (3.9%) of our cases (Table 1). EAEC was significantly more frequent in patients with acute diarrhea than in controls ($P < 0.01$).

Table 1 Clinical symptoms and other characteristics of 102 patients with acute diarrhea at hospitals or clinics in Karaj, Tehran, Iran

Clinical and other characteristics	Number	Percent in cases
Diarrhea:		
Frequency[a]	6.47 ± 2.16	–
Watery	46	45.5
Mucus	59	42.2
Blood	7	6.9
Vomiting	31	69.6
Anorexia	19	18.6
Lethargy	62	60.8
Abdominal pain	6	5.9
Fever[b]	4	3.9

[a]Mean ± SD number of loose or watery stools per 24 h.
[b]Temperature more than 38.5°C.

Conclusion: These results suggest that EAEC should be considered as an important diarrheal pathogen among children under 5 years of age in Karaj, Iran.

Helicobacters – Update 2003 (Symposium arranged by EHSG)

S92 Lipopolysaccharides of *Helicobacter* spp. characteristics and role in pathogenesis

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Like other Gram-negative bacteria, *Helicobacter* spp. contain lipopolysaccharides (LPSs), a family of phosphorylated glycolipids, in their outer membranes. In the human gastroduodenal pathogen *Helicobacter pylori*, LPSs have been the subject of intensive investigation as pathogenic factors. *H. pylori* LPS has low immunological activities, attributed to the unique structure of its lipid A component, and is considered to contribute to the ability of the bacterium to chronically colonize the gastric mucosa. On the other hand, differences in core oligosaccharide (COS) structure of the LPSs has been implicated in the induction of pepsinogen and laminin interaction, as well as the virulence of strains particularly those associated with duodenal ulcers. Nevertheless, serological studies have shown that, compared to enteric bacteria, there are epitopes in the COS that are conserved and unique to *H. pylori* LPS, which may represent candidate epitopes in immunodiagnostic or vaccine development. Furthermore, *H. pylori* LPSs mimic Lewis (Le) blood group determinants, particularly Lex and Ley, in their O-chains, but the role of this mimicry has been a matter of intensive debate. Nevertheless, evidence exists that this mimicry can influence colonization in the gastric mucosa by (i) Lex/Ley aiding camouflage of the bacterium in the mucosa or (ii) Lex acting as a bacterial adhesin. On the other hand, chronic infection by *H. pylori* can lead to a breakdown in immunological tolerance and LPS-expressed Lex/Ley have been implicated in the development of autoreactive antibodies against the gastric proton pump and in the development of atrophic gastritis. More recently, investigations have been performed on the LPSs of non-*H. pylori* gastric (*H. felis*, *H. canis*, *H. musteale*, *H. bizzozeronii*) and enterohepatic (*H. hepaticus*, *H. bilis*, *H. pullorum*, *H. rappini*) helicobacters. *H. mustelae* and *H. hepaticus* produce low-, whereas the others produce high-molecular-mass LPSs. Also, unlike *H. pylori* (with Le antigens) and *H. mustelae* (with blood group A), no similar molecular mimicry was observed in the other LPSs. Furthermore, a general absence of serological cross-reactivity with the core of *H. pylori* LPS was observed indicating the occurrence of differing COS which could be exploited in immunodiagnostics. Furthermore, detailed chemical analysis of the LPSs has revealed the occurrence of novel sugars and distinctive sugar compositions which could prove of diagnostic value.

The European Centre for Control of Infectious Diseases: what model will meet the challenge

S95 The European Centre for Infectious Diseases: a project at the size of Europe's needs and historical mission

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The European Centre for Infectious Diseases (ECID) has been proposed 6 years ago (1–2) to face the threat of emerging and reemerging infectious diseases. Inspired from the US Centers for Disease Control, it aims at settling in Europe a centralized structure with walls, relayed by outstations and corresponding centers, all Internet-connected. The missions of the ECID would be 3-fold: (a) path-breaking, holistic research avoiding redundancies with the research programs of the existing centers; (b) centralized surveillance and control respecting the national sovereignties; (c) professional trainings. The ECID should look for the active participation of Eastern Europe, former USSR and Turkey, and should establish privilege links with developing

countries ('microbes ignore borders'). The project has been challenged by the supporters of the 'virtual CDC' (electronic networking of the existing centers (3–5); a position until recently retained by the European Parliament (5). The bioterrorism threat led the Parliament to adopt the concept of a centralized structure (6–7). However, the project still is too shy, and even if it goes in the right direction, will not be enough to efficiently control major bioterrorism threats and epidemics. Research and collaborative strategies are proposed to make the ECID an efficient tool toward reaching these goals (8).

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Antifungal immunotherapy (Joint symposium arranged with the ICHS)

S97 Adjunctive cytokine therapy against moulds: where do we stand?

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Invasive infections due to filamentous fungi (IFIs) have become a major cause of increased morbidity and mortality in immunocompromised patients. Genetic and acquired (disease- or therapy-related) risk factors predispose to IFIs. Factors that modulate the host immune response against molds are hemopoietic cytokines [granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF) and macrophage CSF (M-CSF)], Th1 cytokines [interferon-gamma (IFN-gamma), interleukin (IL)-15], Th2 cytokines (IL-4 and -10) and other cytokines. Hemopoietic cytokines have been shown not only to enhance the phagocytic function against several pathogenic molds but also to increase the number of phagocytes in vivo. While Th1 cytokines do not increase the number of phagocytes, they have been generally shown to enhance their antifungal activity against *Aspergillus* spp. and other pathogenic molds. Th2 cytokines have been found to suppress the antifungal activity of phagocytes. The beneficial effects of certain hemopoietic and Th1 cytokines have been translated to some degree of favorable outcome of invasive aspergillosis in immunocompromised animal models. In addition, neutralization of Th2 cytokines improves outcome of murine invasive aspergillosis. Cytokines may be administered to patients in different stages of their disease: prophylactically at the beginning of granulocytopenia to shorten its duration, preemptively at the onset of febrile granulocytopenia and therapeutically upon diagnosis of a probable or definite IFI during granulocytopenia. In addition, they can be given as prophylactic or therapeutic intervention in high-risk nongranulocytopenic hosts. Transfusions of granulocytes from healthy donors are another mode for immune reconstitution of profoundly granulocytopenic patients with difficult-to-treat mold infections under investigation. Hemopoietic cytokines or IFN-gamma combined with antifungal chemotherapeutic agents such as polyenes, newer azoles or echinocandins as well as hemopoietic cytokines combined with granulocyte transfusions appear to have enhanced effectiveness against clinically relevant molds. Anecdotal case reports and a few nonrandomized clinical studies support use of certain cytokines in prevention or treatment of IFIs. However, randomized studies with the specific aim to examine the impact of cytokines on IFIs remain to be performed.

S98 Host defense mechanisms against fungal infections: a comprehensive perspective

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There are multiple defense mechanisms that function cooperatively in the host response to fungi. As a result, infections with these organisms are mild and self-limiting in hosts with an intact immune system. The normal defense mechanisms involved are multifactorial, but may be classified as either innate or acquired resistance. Innate mechanisms act immediately and are the first line of defense against fungi. These include natural barriers such as intact skin, which protects against infection caused by fungi that colonize the skin, or intact epithelial linings of the respiratory, gastrointestinal and genitourinary tracts that also act as mechanical, microbiological, and chemical barriers to fungi. Circulating proteins such as transferrin are inhibitory to the growth of some fungi, including *Candida albicans*, Histoplasma capsulatum, and members of the Mucorales family. While many fungi are resistant to lysis by terminal components of the complement system, factors released in the complement cascade are important in resistance to infections with *C. albicans, Aspergillus fumigatus*, and *Cryptococcus neoformans*. Phagocytic cells, including polymorphonuclear leukocytes, monocytes, macrophages, eosinophils and dendritic cells are an extensively studied, critical component of the innate response to fungal infections. Oxygen radicals, peroxides, nitric oxide and cationic proteins are generated by these cells in varying degrees in response to different organisms, and may result in their inhibition, damage, or death. Further activation of these phagocytic cells is accomplished by release of proinflammatory cytokines and chemokines by T cells, as well as other phagocytes. Production of regulatory cytokines is a critical component of acquired resistance to systemic fungal infections. The pattern of release of these proteins may distinguish between increased resistance and susceptibility, with a strong Th1 response providing protection and a strong Th2 response resulting in increased disease severity. While antibodies may play a beneficial role in cryptococcosis and candidiasis, their overall importance in other fungal infections remains unclear. Thus, the innate and adaptive immune systems are finely tuned arms of the host response that cooperate extensively in controlling and/or preventing fungal infections.

Prevention of surgical infections (Symposium arranged with the ESGNI)

S100 Recent developments to prevent surgical infections

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Objective: To evaluate recent developments in the prevention of surgical infections.
Methods: Review of the literature.
Results: The most interesting developments have been made regarding the preoperative preparation of the patient. Especially, the strong effects of preoperative warming of the patient at whole or the incision site locally on the SSI rate are impressive and warrant more investigations. Also, the effects of strict control of the perioperative blood glucose levels and of preoperative eradication of nasal carriage of *S. aureus* have resulted in lower surgical infection rates and should be studied in more detail.
Conclusions: The protective effect of local and systemic warming before the surgical procedure has been described twice in well-designed studies. Although a final judgment about the effectiveness can only be made after more extensive investigations, there seems little against this cheap and save measure. Therefore, in settings with a high SSI-rate the implementation of preoperative warming can be justified. Before widespread implementation

of preoperative eradication of nasal carriage of *S. aureus* can be promoted more extensive investigations should be performed.

S101 Surveillance of surgical site infections in abdominal surgery

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Background: Surgical site infections (SSI) have a clear impact on morbidity, mortality and costs. They represented >25% of all nosocomial infections found in Swiss hospitals during prevalence surveys organized by the Swiss–Noso network in 1996, 1999, and 2002 (asymptomatic bacteriuria not included). Surveillance, including feedback of their SSI rates to surgeons, has been shown an effective preventive measure.
Methods: In 1998, five community hospitals and one tertiary-care university hospital in Western Switzerland initiated a surveillance program based on the principles of the US National Nosocomial Infections Surveillance (NNIS) system with the objectives to reduce the rates of SSI, to develop a quality indicator, and to better understand the epidemiology of SSI in these settings.
Results: As of December 31, 2001, 7368 patients who underwent abdominal or thoracic surgery had been included. A follow-up at one month was available for 96% of them. Overall, 601 patients (8.2%) suffered from SSI (42% superficial incisional, 28% deep incisional, 30% organ or space infections). Two hundred and four of them (34%) were diagnosed postdischarge. Two hundred and forty-two (40%) had to undergo percutaneous or surgical drainage. Seventy-one (12%) were re-admitted for the treatment of their infection. SSI rates varied between interventions from 2% (herniorrhaphy) to 19.9% (colon surgery), and from 5.2 to 10.5% between hospitals. Multivariate analysis showed that the three variables included in the NNIS index did significantly predict SSI in digestive surgery (contamination class, OR = 1.6 [1.5–1.8]; ASA score, OR = 1.4 [1.2–1.6]; duration > T, OR = 1.9 [1.5–2.4]), together with other variables such as the number of procedures done during the intervention (>1 procedure, OR = 1.5 [1.2–1.9], or a re-intervention for a non infectious complication (OR = 3.0 [1.9–4.6]). The use of a laparoscope was found protective (OR = 0.4 [0.3–0.5]). Odds ratios adjusted for the NNIS index and allowing intersurgeon and interhospital comparisons were periodically provided to surgeons or infection control committees, respectively. This program motivated specific measures such as the implementation of the 1999 CDC guidelines for the prevention of SSI. SSI rates in the different hospitals were consistently lower in 2001 than in 1998.
Conclusion: Multicentric surveillance of SSI according to the NNIS principles proved suitable and useful in the Swiss setting, at least in digestive surgery.

Emerging flea-borne rickettsioses

S103 Flea borne bacteria and bacterial diseases

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In developed countries human beings are exposed to pet fleas (cat and dogs) as exposure to rat fleas dramatically decreased. Several emerging diseases have been associated with *Ctenocephalides felis* the cat flea. The bacteria found in cat fleas are *Bartonella quintana*, *B. henselae*, *B. clarridgeiae*, *B. koehlerae*, *Rickettsia felis* and *R. typhi*. *B. henselae* is the agent of cat scratch disease in human beings. It can also cause bacillary angiomatosis, retinitis, meningoencephalitis and endocarditis. It is associated with chronic asymptomatic bacteremia in cats. It is transmitted to humans by cat scratch or bite and sporadically by fleas. *B. quintana* is the agent of human diseases including trench fever, bacillary angiomatosis, endocarditis and chronic bacteremia. Some cases have been associated with cat fleas. There are no clear evidence of human diseases caused by *B. clarridgeiae* or *B. koehlerae* which causes chronic bacteremia in cats. *R. typhi* is the agent of murine typhus, it is usually transmitted by rat fleas. It was identified on *C. felis*, obtained from opossums, in California. *R. felis* is a new worldwide agent of a new spotted fever. It was found in fleas from America, Africa and Europe. Human cases were reported in Europe and America. In conclusion, pet fleas represent a threat for human health.

Viral pathogens in water: a revived threat

S104 Caliciviruses: like a fish in water

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Noroviruses, belonging to human caliciviruses, are largely recognized as the major cause of foodborne outbreaks. Their dominant role has also been documented in community-based studies on gastrointestinal disease. Thus noroviruses are also the most abundant of the human pathogenic viruses released in the environment. Their resistance to physicochemical stress facilitates their survival in the environment. The large number of distinct noroviruses together with a shortlived immunity elicted by the infection makes the population susceptible for repeated outbreaks. Both vomit and stool of a norovirus victim is highly infectious. Person-to-person transmission is considered most common, but spread via surfaces, food or water is increasingly recognized. The bulk of the excreted virus is released into sewage. The processes used in sewage treatment allow in many instances the virus to pass infectious into receiving water bodies. Thus noroviruses are well suited for waterborne spread. There are several ways by which contaminated water may reach us. The 'classical' route is through bivalve molluscs, mainly oysters. Their role as hepatitis A virus source has been well established and is still a serious threat. Noroviruses are also well suited for this spread and *trans*-continental outbreaks have been described both in USA and in Europe due to widely distributed oysters from a single contaminated bed. The role of fresh produce contaminated through irrigation as a source of norovirus outbreaks is not well documented. Based on epidemiological evidence frozen berries, especially raspberries, have commonly been connected to outbreaks. Here also multinational outbreaks have been indicated. Recreational use of water is apparently also a way to catch a norovirus infection. Swimming pools and spas are at risk of contamination. The most efficient way of delivering the virus is through drinking water. We have earlier reported on a major waterborne norovirus outbreak in Finland comprising about 2500 diseased in a community of 5000 inhabitants (Kukkula et al. J Inf Dis 1999; 180: 1771–1776). Since then, we have obtained patient and water samples from altogether 28 outbreaks. Of these, 18 were caused by noroviruses as evidenced by virus in patient stool samples. In eight of the above mentioned waterborne outbreaks the norovirus could be detected in water samples, too. In all except, one case drinking water was the suspected source of infection.

S105 Hepatitis A and other viruses in shellfish

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Human pathogenic viruses accumulate within the edible tissues of shellfish grown in sewage contaminated waters. Current standards rely solely on bacteriological parameters and do not ensure the prevention of shellfishborne viral gastroenteritis and hepatitis. Among the causative agents of these shellfishborne diseases, Norwalk-like viruses (Norovirus) and hepatitis A virus (Hepatovirus) are the most relevant in terms of occurrence and severity, respectively. Recently, an outbreak of hepatitis A, affecting 183 people, was caused by consumption of coquina clams, imported frozen from Peru. Hepatitis A virus and other enteric viruses were detected in this shellfish which met all EU standards. F-specific RNA bacteriophages are proposed as alternative indicator microorganisms. Yet, exhaustive work is required to ascertain their validity, since discordances between the occurrence of these surrogates and that of actual viruses in shellfish has been reported. In the

meantime, the safest approach for the prevention of shellfishborne viral diseases, is to monitor for the presence of Norwalk-like viruses and hepatitis A virus in shellfish and shellfish growing areas. Currently available molecular procedures provide tools for the specific and sensitive detection of viral pathogens in shellfish, thus enabling a safer evaluation of its virological quality. However, quality control and quality assurance issues must be solved, as well as simplification and automation, before molecular procedures could be adopted by routine monitoring laboratories.

Cellular transport of antimicrobials

S107 Mammalian peptide transporters as targets for delivery of antimicrobials and other drugs

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Cellular uptake of amino acids in form of di- and tripeptides occurs in mammals by the two plasma membrane peptide transport systems PEPT1 and PEPT2. PEPT1 is mainly expressed in the apical mem-brane of epithelial cells of the small intestine and is responsible for the absorption of dietary di- and tripeptides and structurally related drugs such as selected β-lactam antibiotics, peptidase and protease inhibitors as well as designed prodrugs. PEPT2 in contrast shows a more widespread expression within the organism. In kidney, it mainly mediates the reabsorption of filtered di- and tripeptides and peptidemimetics, but its role in other tissues such as lung, mammary gland, choroid plexus, glia cells and others has not yet been determined. Both transporters have been characterized as proton-dependent and electrogenic by coupling of peptide movement across the membrane to movement of protons down an inwardly directed electrochemical proton gradient. We are expressing the transporters in yeast cells and *Xenopus oocytes* and analyze function by flux studies and electrophysiological methods. As the transporters accommodate thousands of different peptides as well as a large number of drugs, we systematically analyzed the structural requirements for binding and transport. The current state on understanding of the structure-affinity relationship will be presented. For drug delivery purposes a new screening system has been developed that allows compounds to be identified easily that utilize the transporters for uptake into tissues that provides a high bioavailability. Most recently, we have also established lines of transgenic *C. elegans* and mice that allow analysis of the phenotypical consequences of gene knock out encoding the corresponding peptide transporters in these animals and by use of GFP- and LacZ-constructs a detailed analysis of sites of gene expression was performed.

S108 Efflux transporters and HIV drugs in the brain

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Introduction: The brain is a sanctuary site for HIV and contributes to the reinfection of the whole body from the brain. Therefore, anti-HIV drugs, particularly protease inhibitors (PI's), have to enter the brain to inhibit the replication of the virus there. However, several anti-HIV drugs are substrates for efflux transporters.
Objective: To study the role of efflux transporters in the transport of the PI's (amprenavir, ritonavir and indinavir) HIV drugs across the in vitro blood–brain barrier (BBB).
Methods: For the in vitro studies, we have used a BBB model comprising a coculture of bovine brain capillary endothelial cells and rat astrocytes. In addition, the LLC-PK1 wildtype, the MDR1-transfected LLC-PK1, the MRP1-transfected LLC-PK1 and the Caco-2 cell lines were used to study the involvement of efflux transporter in the transport of PI's.
Results: The data indicate that in the BBB model amprenavir, ritonavir and indinavir showed transport polarity. The inhibitors LY335979, verapamil and SDZ-PSC 833 were able to reduce this transport polarity indicating that these PI's are substrates for the P-glycoprotein (Pgp) cation transporter. Similar data were obtained in the MDR1- transfected LLC-PK1 cell line and in the Caco-2 cell line. Surprisingly, in the MRP1-transfected cell line probenecid reduced transport polarity of ritonavir and indinavir indicating that these compounds are also substrates for the MRP1-anion-transporter.
Conclusions: Amprenavir, ritonavir and indinavir are substrates for Pgp. In addition, ritonavir and indinavir are also substrates for the MRP1-efflux transporter.

Emerging infectious diseases

O109 Emerging tick-born zoonoses in Eastern Croatia

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Objectives: Specific IgG antibody titers to *Ehrlichia chaffensis*, HGE agent, *B. divergens*, *B. burgdorferi* and rickettsia species in group of 100 hospitalized and unhospitalized patients, who have experienced tick bite, were examined.
Methods: A serological testing was performed in group of about hundred persons, who have experienced tick bite. About on half of them were hospitalized (some of them for TBE, most of them for LB), other half visited their GP, because of treat of developing some of tick-born-diseases. A serological testing was performed by commercial ELISA test for LB, and IFA test for HGE agent, *E. chaffensis*, *B. divergens* and rickettsia species and using reconvalescent sera, taken about 3 weeks after tick bite, or after admitting to hospital. Examined persons differ by occupation, place of living, age and sex.
Results: There are still no data of circulation of these zoonotic agents in our area, and our goal was only to find out is there any sign of this circulation in the Eastern Croatian countries. We found three *B. divergens* positive sera, five HGE agent positive sera, two *E. chaffensis* positive sera, five rickettsia species positive sera (four RMSF group, one tiphus group), and 32 LB positive sera (all patients who were hospitalized for LB). Total number of sera is small, so we did not made any statistical analysis. Of course, all similar investigations like, we have had a huge number of persons with possibility of high exposure to tick bite.
Conclusion: In the Eastern Croatian countries, we found sera from persons who experienced tick bite seropositive on HGE agent, *E. chaffensis*, *B. divergens*, rickettsia species and LB agent. That is way more detailed investigations of reservoirs, vectors and patients must be provided.

O110 Emerging infections due to nonfermenter bacilii

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Introduction: Non fermenter bacilii are ubiquitous in the environment such as soil, water, plants and decaying organic material including foodstuffs. They are found in all moist areas in the hospitals where they can resist most of the common disinfectants. They can cause significant infections in the immunocompromised. Kenya is currently in the grip of HIV/AIDS pandemic with 60% hospital admissions being HIV positive. In the Aga Khan hospital Nairobi, several clinical isolates are found to be nonfermenter bacilii from these patients. Currently internationally accepted guidelines are not available for the antimicrobial sensitivity testing of several members of this group of bacteria.
Methods: Clinical microbiology lab at the Aga Khan Hospital is a fully equipped modern facility for carrying out all standard bacteriological procedures. Blood cultures are performed by Bactec9060 (Becton Dickinson). Gram negative bacilii are identified by API system (Biomerieux). API20NE is used for nonfermenter bacilii. Identifications were confirmed whenever

necessary at the center for microbilogy research at Kenya Medical Research institute. Results over the past 3 years were compiled for this report.
Results: Around 10960 Gram negative rods were isolated during the study period out of which 1041 (9.5%)were nonfermenters. Eight hundred eighty-four (85%) comprised of *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Ps. putida*. Interestingly, rare isolates like *Ralstonia picketii*, *Sphingomonas spiritivorum*, *Cryseomonas meningosepticum*, *Ochrobacterium anthropi*, *Photobacterium damsela* and *Agro bacterium* species were isolated from significant clinical infections. Ninety-six nonfermenters could not be identified since the API profiles were not found in the data bank. Majority of the isolates were multi drug resistant to most of the broad spectrum Antibiotics when tested by the NCCLS disc diffusion protocol. Around 80% of the patients suffering these infections had highly complicated clinical course despite broad spectrum combination antibiotics regimens.
Conclusions: Better methods of identification are required for scientific documentation of these infections. Guidelines on clinical management and in vitro sensitivity testing of the isolates are urgently required for saving the life of the immunocompromised.

O111 Seroprevalence of Crimean–Congo hemorrhagic fever in the Sistan va Baluchestan province of Iran

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Objectives: Since 1999, there have been more than 137 confirmed Crimean-Congo Hemorrhagic fever (CCHF) cases from different parts of Iran. There is no idea about the real prevalence of this disease in Iran. The main objective of this study was the determination of the CCHF seroprevalence rate, using specific ELISA methods, in the Sistan va Bluchestan province which has the highest number of CCHF cases in Iran.
Methods: In this cross-sectional study, 300 people were sampled using probability proportional to size cluster sampling method from the general population of Zabol and Zahedan districts located in the northern half of the province. In addition to blood sampling of each subject, the following variables were checked for them through a questionnaire: personal information (age, sex, job), past history of tick bite, contact history with livestock, history of slaughtering, presence of an animal place at home.
Results: The results showed that only seven (2.36%) of the subjects were positive for anti-CCHF IgG. The most important risk factor for antibody positive subjects were those related to having or taking care of domestic animals at home ($P = 0.020$). The education level of the antibody positive subjects was significantly lower ($P = 0.005$). None of the antibody positives people had history of tick bite or contact with a confirmed or suspected case of CCHF, however, all of them have history of contact with livestock. Three of the antibody positives were Afghan refugees inhabited in these districts.
Conclusion: The CCHF Seroprevalence rate in Sistan va Bluchestan province was lower than our expectations. This study reveals some of the behaviors and life styles that predispose persons to the disease. We hope to find more information through another study, which has been sponsored by WHO (EMRO) in the same setting and with 6 months follow-up for the determination of the CCHF incidence rate in the Sistan va Bluchestan province.

O112 The efficacy of oral Ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Iran

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Crimean-Congo Hemorrhagic fever (CCHF) is a lethal hemorrhagic fever caused by a tick-borne virus. There are few reports on the efficacy of oral ribavirin in the treatment of CCHF patients. This study was designed as a historical cohort with 187 clinically suspected cases since June 1999 to the end of September 2001. Only 139 of 187 suspected cases were treated with oral ribavirin based on the availability of the drug. A total of 81 of 187 cases were serologically confirmed to have the disease. The two groups (treated and nontreated) were compared for the incidence of outcome (Survival). Ninety-seven (69.8%) of 139 suspected treated cases and 61 (88.4%) of 69 confirmed treated cases were survived. Based on this study, the efficacy of oral ribavirin is measured equal to 80% and 34% in confirmed and suspected groups, respectively. We conclude that oral ribavirin is an effective treatment for hemorrhagic form of CCHF patients. We should be aware of the limitations of observational studies. However, there is no randomized control trial in the literature, and it can not be performed in future due to medical ethics. Therefore the results of this study could provide a valuable information in deciding how to treat CCHF patients

Bacterial pathogenesis

O113 Occurrence of the enterococcal surface protein Esp and in vitro adhesion properties of vancomycin-susceptible *Enterococcus faecium* isolates of different origins

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Background: The enterococcal surface protein, Esp, has been suggested to have a role in enterococcal virulence.
Objectives: To determine if vancomycin-susceptible *Enterococcus faecium* (VSE) isolates of different origin, i.e. blood culture isolates, normal intestinal microflora, and probiotic products, differ in the occurrence of the *esp* gene and in their ability to adhere to gut the mucosal cells.
Methods: A total of 61 *E. faecium* isolates – 29 derived from blood cultures of patients hospitalized in Sweden, 30 from the normal intestinal microflora of healthy Swedish volunteers, and two different probiotic strains – were investigated. An inoculum of approximately 107 viable bacteria were added to a confluent grown human colon cancer cell line (Caco-2) and incubated for 2 h. Nonadhered bacteria were washed away and the number of adhered bacteria were determined with viable count after lysis of the eukaryotic cells. Carriage of the *esp* gene was detected by PCR using *esp*-specific primers.
Results: There was no difference between the groups of isolates in their ability to adhere to the Caco-2 cells. Approximately half of the infection-derived *E. faecium* isolates carried the *esp* gene, while none of the probiotic strains and only two of the normal microflora isolates were positive for the *esp* gene.
Conclusions: Clinical isolates and normal microflora isolates of *E. faecium* seem to adhere equally well to Caco-2 cells. Presence of the *esp* gene does not seem to affect the adhesion of *E. faecium* strains to these epithelial cells. Enrichment of *esp* in the clinical isolates indicates that *esp* might be of importance to virulence in *E. faecium*.

O114 The autolysin atlE is possibly involved in the maintenance of in vivo foreign body infections in *Staphylococcus epidermidis*

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Objectives: Foreign body-associated infections (FBI) are a major cause of morbidity and mortality. During FBI, an initial adhesion of the bacteria is followed by accumulation and biofilm formation. The atlE encodes glucosamidase and amidase involved in peptidoglycan cleavage during cell division in *Staphylococcus epidermidis*. AtlE-negative mutants have a reduced virulence in in vivo models for FBI and a decreased adhesion to polystyrene in vitro. The current study aims to evaluate the expression of the *atlE* gene during in vitro and in vivo foreign body-associated growth.
Methods: The expression of atlE was quantified in *S. epidermidis* with RT quantitative PCR as the cDNA/gDNA quotient as previously described (BBRC, 2002). AtlE expression was followed over time in vitro in NaCl 0.9% in planktonic (104 samples) and sessile (104 samples) bacteria and in vivo in a rat model for foreign body infections in sessile bacteria (293 samples). In vitro time points were 0, 10, 35, 60, and 180 min. In vivo time points were 0 and 15 min, 1, 2, 4, 6, and 12 h, and 1, 2, 7, and 14 days.
Results: In vitro inoculation in NaCl 0.9% induced a decrease in atlE expression in sessile and planktonic bacteria ($P < 0.0001$; one-way ANOVA). This decrease was slower and less pronounced in the sessile group ($P = 0.002$;

two-way ANOVA). In vivo implantation of the infected catheter induced a small nonsignificant increase in atlE expression that peaked at $t = 60$ min, followed by a nonsignificant dip at $t = 1$ day. Expression levels after 2 h were identical as those after 1 and 2 weeks. The net evolution of atlE expression was given by log10 (atlE expression) = $(-1.372) + (-7.08 \times 10^{*}6) \times$ time, with a $R^2 = 0.01$. These constant expression levels contrast with those observed in many other genes studied.

Discussion: Our data do not support an important role of the atlE in the initial adhesion of *S. epidermidis* to polyurethane catheters. The constant expression levels of the *atlE* gene throughout the course of in vivo FBI however suggest, but do not prove a role (primary or secondary), in the maintenance of in vivo FBI.

O115 The influence of antibiotics on the production of shiga toxins

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Background: The clinical and public health importance of infection by shiga toxin-producing *Escherichia coli* (STEC) have become increasingly recognized internationally since the early 1980s. STEC is associated with human disease including mild diarrhea, severe bloody diarrhea, and hemolytic uremic syndrome (HUS). Because antimicrobial agents may play a role in the pathogenesis of severe STEC disease, chemotherapy for STEC infections or complications remains controversial. We report three cases of HUS caused by Stx2-positive STEC. The patients were treated by cephalosporins because of complications (respiratory tract infection). We found that cephalosporins were able to enhance production of shiga toxin 2 in vitro.

Methods: The clinical STEC strains were incubated with ampicillin/sulbactam, meropenem, cefepime, piperaciline, piperacilline/tazobactam, cefotaxime, ceftazidime, gentamycine, amikacin, ciprofloxacine, and trimethoprime/sulfamethoxazole using dilution method. After incubation, the shiga toxin was separated and the presence of shiga toxins was confirmed by ELISA with monoclonal antibodies against Stx2. We used Mann–Whitney *U*-test for statistical analysis.

Results: On the basis of ELISA tests, we have found that ciprofloxacin, trimethoprime/sulfamethoxazole ($P < 0.001$), amikacine, gentamycine, and cefepime ($P < 0.01$) and cefotaxime, ceftazidime, piperaciline, piperacilline/tazobactam ($P < 0.05$) significantly induced the production of shiga toxin 2. Shiga toxin production was not influenced by meropenem and ampicillin/sulbactam.

Conclusions: It is important not only to isolate STEC from HUS patients but also to detect the influence of antibiotics which are used in hospitals (and were given in cases reported in this study) to shiga toxin production. On the basis of our results, the physicians disrupted cephalosporine therapy. All three patients suffering from HUS have survived with no serious sequelae.

O116 Characterization of the penetration ability and cytotoxicity of *Serratia marcescens*

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Objectives: To characterize the penetration ability and cytotoxicity of clinical isolates of *Serratia marcescens* that caused either invasive or noninvasive infections.

Methods: Nineteen each of blood and urinary isolates of *S. marcescens* were examined in vitro for their ability to penetrate a Madin–Darby canine kidney (MDCK) epithelial cell monolayer by using a membrane filter system. Bacteria were inoculated at a ratio of 100 : 1 (bacteria:cell) to the apical surface of MDCK cell monolayer. The viable bacteria in the basolateral medium were plate-counted at several time intervals. The cytotoxicity against MDCK cells was assessed by measuring the concentration of lactose dehydrogenase (LDH) released from the cells into the medium. The Chi-square test was used for statistical analysis.

Results: Significantly more blood than urinary isolates were detected in the basolateral medium after 6 h (18 vs. 10; $P < 0.01$) incubation. Nine blood isolates compared to only two urinary isolates were high penetrators (>1 000 0000 CFU/mL; $P < 0.05$). A similar significant difference in cytotoxicity between the two groups was also found (17 blood vs. 9 urinary; $P < 0.05$). The average LDH level of all blood isolates at 6 h after inoculation was 157 ± 74 U/L (median, 168 U/L) and that of all urinary isolates was 92 ± 63 U/L (median, 58 U/L). LDH level increased proportionally as the number of bacteria that penetrated increased, indicating a close association of the penetrative ability of bacteria to their cytotoxicity. By using a fluorescent acridine orange-crystal violet staining method, viable bacteria were observed intracellularly at 2 h, and the amount increased at 3 h, indicating the invasive ability of *S. marcescens* and a possible mechanism of transcytosis penetration. Further characterization of the cytotoxicity indicated that viable whole bacteria were required to induce cytotoxicity in vitro, and neither toxic exoproducts produced during growth nor intracellular materials were responsible for the cytotoxic effect. Inhibition of RNA and protein synthesis abolished the cytotoxicity as well as the penetration.

Conclusions: Although other mechanisms may be involved, the abilities to penetrate epithelial barriers and the associated cytotoxicity are important virulence factors contributing to invasive infections associated with *S. marcescens*.

O117 Mucoidy in *Stenotrophomonas maltophilia* – a potential virulence factor in cystic fibrosis-associated lung infection?

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Background: *Stenotrophomonas maltophilia* (Sm) is an emerging pathogen in cystic fibrosis-associated lung disease, but putative virulence factors of the bacterium are not well-characterized. *Pseudomonas aeruginosa* (Pa) is an established pathogen in CF and production of alginate by Pa (resulting in a mucoid phenotype) is recognized to play a key role in the pathogenesis of pulmonary infection. The isolation of a mucoid strain of Sm prompted an investigation to identify factors which might regulate expression of mucoidy by Sm.

Methods: Six strains of Sm of clinical and environmental origin, including mucoid strain E13 and two control strains of Pa, were used. The stability of the mucoid phenotype (MP) was investigated by repeated subculture on to blood and nutrient agar at 20, 30, and 37°C (as antibiotic resistance phenotype and exoenzyme production in Sm is associated with growth temperature). Expression of mucoidy in Sm strains was determined following growth under conditions of increased osmolarity (media supplemented with 0.5, 1, 2, and 5% w/v NaCl), dehydration (media supplemented with 0.5, 1, 3, 4, 5,7, and 10% v/v ethanol), and nitrogen and phosphate limitation (N and AP media) – all of which induce mucoidy in Pa. To determine whether the genes for mucoidy in Sm were plasmid-associated, plasmid extraction using commercial and in-house methods were used.

Results: Mucoidy persisted in strain E13 after 20 serial subcultures, irrespective of the growth medium/temperature; however, optimum conditions for mucoidy were growth on blood agar at 30°C for 48 h. Increased osmolarity was associated with onset of mucoidy in two isolates most notably at 2% NaCl. Dehydration was associated with production of a mucoid phenotype in one strain at a range of ethanol concentrations at 20 and 30°C. Growth on N and AP media was not associated with mucoidy. The failure of AP medium to induce mucoidy suggests the MP it is not associated with alginate. Plasmids were not detected in strain E13.

Discussion: Mucoidy in strain E13 appears a stable phenomenon. Although a limited number of strains were tested, mucoidy could be induced under conditions of increased osmolarity and dehydration at lower growth temperatures. Mucoid strains of Sm from clinical and environmental sources are extremely rare, but as most laboratories incubate sputum and other specimens at 37°C, these strains may go undetected and the importance of the mucoid phenotype might be underestimated.

Vaccines

O118 Additive effect of influenza and pneumococcal polysaccharide vaccine in prevention of hospitalization and mortality in elderly persons

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Objective: The use of influenza and pneumococcal vaccines has been limited in many countries, partly due to uncertainties regarding vaccine effectiveness in the elderly people. The aim of this study was therefore to assess the effectiveness of influenza and pneumococcal vaccination, separately and in combination, in reducing need for hospital treatment and death in an elderly population.
Methods: A prospective population-based intervention cohort study. All individuals 65 years and older ($n = 258\,754$) in Stockholm County, Sweden, were offered vaccination during a 2-month campaign. Data on end-point diagnoses during the follow-up year (December 1, 1999 to November 30, 2000) were obtained from the administrative database in Stockholm County Council. Main outcome measures were incidence of hospital admissions, days of hospital treatment, and deaths due to influenza, pneumonia, invasive pneumococcal disease (IPD), cardiac failure, and chronic obstructive pulmonary disease (COPD).
Results: Vaccination was performed in 124 702 (48%) persons, whereof 72 107 received both vaccines, 29 346 only the influenza, and 23 249 only the pneumococcal vaccine. Compared with the unvaccinated cohort, a tendency for a lower incidence of hospitalization due to influenza, pneumonia, and IPD was seen in persons vaccinated with only one of the influenza, or pneumococcal vaccines. However, vaccination with both vaccines were additive, with a reduction of hospital admissions for influenza (37%), pneumonia (29%), IPD (44%), cardiac failure (12%), and COPD (9%). This reduction was statistically significant for all investigated diagnoses, except for IPD (OR 0.56; 95% CI 0.30–1.05) where the total number of patients was small. In-hospital mortality for pneumonia (OR 0.65) and cardiac failure (OR 0.64) was significantly lower in those who received both vaccines.
Conclusions: Vaccination with influenza and pneumococcal vaccines together was effective in reducing need for hospital admission and deaths from influenza and influenza-related diseases. Our data also support that pneumococcal vaccination alone is about 70% effective in prevention of IPD in the elderly, and indicate that the vaccine may be effective in preventing pneumonia overall, although to a low degree.

O119 Identification and immunogenicity of a naturally processed measles virus peptide eluted from class II HLA-DRB1*0301

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Objectives: The identification and characterization of antigenic epitopes of infectious pathogens by $CD4^+$ T-cells has been a major research interest. The rapid characterization of defined peptides that are critical to viral immunity, including measles, has been significantly enhanced by mass spectrometry (MS). Hence, we can describe for the first time the direct identification of class II HLA-DRB1*0301 (DR3) measles-derived peptide from measles virus (MV)-infected EBV-transformed B cells.
Methods: We developed a B-cell line derived from a measles seropositive human individual homozygous for the *HLA-DR3* allele. We infected EBV-B cells with live MV (Edmonston strain) at a multiplicity of infection of 1 PFU/cell. We purified peptides associated with the DR3 molecules by immunoaffinity and obtained peptide sequences by nano-scale reversed phase HPLC coupled to tandem MS (nano-LC/MS/MS). We evaluated recall immune responses by measuring MV and measles peptide lymphoproliferation.
Results: A class II, 19 amino acid peptide derived from a single measles phosphoprotein (P), ASDVETAEGGEIHELLRLQ, exhibited the capacity to stimulate peripheral blood mononuclear cell responses. The MV-P epitope (residues 179–197) sharing the HLA-DR3 binding motif was recognized by T-lymphocytes of healthy subjects ($n = 95$, aged 11–18 years) who were previously immunized against measles. MV-stimulation indices (Sis; median 4.1, range 0.5–29.1) were generally higher than measles P peptide SIs. Sixteen of the 95 subjects (17%) had P-lymphoproliferation SIs greater than 3.0 (median 1.4, range 0.5–20.3), indicating peptide antigenicity and recognition. Among the 60 subjects who responded to MV stimulation, 12 also responded to the measles P peptide (Spearman's correlation coefficient = 0.38; $P < 0.001$). We observed no proliferation in healthy subjects to randomly chosen measles fusion peptide from the MV proteome.
Conclusions: These data provide direct evidence that measles-derived antigenic peptides were processed by antigen-presenting cells, presented in the context of HLA class II molecules, and were recognized by peripheral blood T-cells from healthy individuals immunized with measles vaccine. The study of cell-mediated immune responses to measles-derived peptides in immune persons should provide significant insight into the design and development of new subunit vaccines.

O120 Protection against leptospirosis after DNA immunization with the hemolysin-associated protein Hap1-encoding gene in gerbils (*Meriones unguiculatus*)

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Background: Leptospirosis is a widespread human and animal disease caused by pathogenic leptospires. The medical and economic losses caused by such forms of the zoonotic disease justify the use of Leptospira vaccines in human or animal populations at risk. However, available vaccines enhance a lipopolysaccharide-directed immune response, which is serogroup-specific. So, these bacterins provide no cross-protection between the different serogroups of pathogenic leptospires.
Objectives: Our previous work determined that immunization with the haemolysis-associated protein Hap1 (designated as LipL32 by Haake) expressed by adenovirus induced in gerbils a significant protection against a virulent Leptospira challenge, while the recombinant protein Hap1 did not induce any significant protection. To avoid the use of adenovirus vector, we checked clinical protection against lethal challenge by DNA vaccination system even if the efficacy of DNA vaccination was recognized essentially in antiviral immunity.
Methods: The *hap1* gene from *Leptospira interrogans* ss serovar autumnalis (aut) and *L. kirshneri* serovar grippotyphosa (grip) was cloned in pCDNA3.1. Gerbils were immunized with plasmid vectors encoding for *hap1* gene under control of cytomegalovirus enhancer–promoter: two intramuscular immunizations with 100 µg of plasmid DNA were performed after a pretreatment by cardiotoxin, controls received DNA empty plasmid in the same conditions. Three weeks after the second vaccination of plasmid DNA, all gerbils were challenged by intraperitoneal injection. Each animal received 10e7 leptospires in 0.5 mL of fresh culture of the virulent *L. interrogans* ss serovar canicola. Gerbils were daily observed, the mortality rate was recorded for 28 days after challenge.
Results: The humoral immune response of gerbils immunized with either pCDNA3.1-*hap1*(grip) or pCDNA3.1-*hap1*(aut) was analyzed by IgG ELISA against the recombinant protein Hap1. Statistical analysis of mortality incidence and survival curves of gerbils were realized: the two groups of animals vaccinated with the protein Hap1 expressed by plasmid were significantly protected against lethal onset of Leptospirosis.
Conclusion: Our results show that the cross-protective effect within pathogenic strains of Leptospira shared by Hap1 protein could be mediated by DNA plasmid vector. This finding should facilitate the design and development of new generations of vaccines against bacteria and especially *L. interrogans* sl.

Betalactamases evolution in Europe: from nature to manufacture

S122 Antibiotic pressure and beta-lactamase selection

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Beta-lactamases probably have a natural role in cell-wall metabolism, but their recent evolution, especially the selection of transferable types, has been predicated by the clinical use of beta-lactam antibiotics. In broad terms, the rise of the new enzyme types has followed the introduction of new beta-lactam classes. Thus, penicillins G and V selected for penicillinase-producing *Staphylococcus aureus*, and the antigram-negative penicillins in the 1960s selected for plasmid-mediated beta-lactamases, principally TEM types, in their target organisms. More recently, oxyimino-cephalosporins have been selected for extended-spectrum beta-lactamases (ESBLs) and AmpC-depressed *Enterobacter* and *Citrobacter* spp., whilst beta-lactamase inhibitors have selected various inhibitor-resistant enzymes and beta-lactamase hyper-producers. Reports of acquired carbapenemases are accumulating, perhaps reflecting the increased use of carbapenems against cephalosporin-resistant strains. It is much less clear why particular enzymes within classes have spread locally, nationally, internationally, or not at all. Why is TEM-1 vastly more successful than any other acquired penicillinase? Why, in the late 1980s and early 1990s, did CTX-M enzymes become the predominant ESBLs of southern S. America, whilst TEM and SHV mutants predominate in Europe, Asia, and the USA? Is the recent spread of CTX-M types in Spain and Poland a harbinger that they will supplant TEM and SHV types in Europe, too. If so, why? Why have so few TEM mutants (but so many more exotic ESBLs) been reported from Japan? And, perhaps most critically, to what extent are carbapenems the primary selectors for the VIM, IMP, and other carbapenemases that are now appearing, allowing that these enzymes are also potent causes of resistance to other beta-lactams, which may also be selectors?

S123 PER and VIM: a local nuisance or more than this?

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During the last two decades, extended-spectrum beta-lactamases (ESBLs) have been disseminated worldwide among the members of the family Enterobacteriaceae. More than 150 enzymes with extended-spectrum activity have been identified so far. Most of these are the mutants of narrow-spectrum beta-lactamases, TEM1-2 or SHV1. These TEM and SHV mutants have been rarely detected among *P. aeruginosa* and *Acinetobacter* species. Recently, however, enzymes with extended-spectrum activity from molecular classes A, B, and D appeared among *P. aeruginosa* and *Acinetobacter.* A molecular 'class A' enzyme, PER-1, was first identified in a *P. aeruginosa* strain in 1993. Later, this enzyme was found to be widespread among nosocomial *P. aeruginosa* and *Acinetobacter* isolates in Turkey. Prevalence of PER-1 producers in other countries, however, remained relatively low. On the other hand, a novel carbapenemase, VIM-1, was identified in Italy. The strain was a carbapenem-resistant *P. aeruginosa* that had been isolated in 1997 from a nosocomial infection. Retrospective analysis of an outbreak in a Greek university hospital, however, revealed the existence of VIM-1 producers in 1996. Three mutational variants of VIM-1 have been identified so far. The members of this novel carbapenemase family have been detected in Korea and Taiwan as well. Since *P. aeruginosa* and *Acinetobacter* are significant nosocomial pathogens, intercontinental spread of carbapenemases and Class A ESBLs among these species deserves interest.

S125 The world of oxacillinases

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When compared to classes A and C, the class D beta-lactamases display very specific properties. They are the smallest serine enzymes and, with regard to their catalytic activity, they are characterized by a fairly large diversity of substrate profiles and by a high hydrolytic activity against oxacillin and cloxacillin. At the genetic level, most of these enzymes are on plasmids or integrons so that their genes disseminate readily in clinical isolates. They are also characterized by a wide variety of amino acid sequences which are all distantly related to the class A and C enzymes (less than 20% of identity). An important subgroup of mutant gene coding for enzymes showing an extended spectrum of activity has been recently identified. Most of the mutant enzymes differ from OXA-10 by a few amino acid substitutions, and are capable of hydrolyzing the later cephalosporins such as ceftazidime. The clinical problems posed by class D beta-lactamases are increased by the fact that these enzymes show very low level sensitivity to inhibition by clavulanic acid. Our knowledge on the catalytic mechanism of the class D beta-lactamases is still limited. Recently, the first crystal structures for class D beta-lactamases were determined (PDB 1EWZ, 1FOF, 1H8Z), showing that the class A, C, and D enzymes share an overall common fold. However, class D beta-lactamases have been shown to form dimers in solution, with maximal activity observed for the dimeric form in the presence of cations such as zinc. Differences adjacent to the active site have been identified in comparison to the two other classes of serine beta-lactamases. For instance, there is no counterpart in class D enzymes to the residues proposed to act as general base in either class A or C (Glu166 and Tyr150, respectively), suggesting that the mechanism by which the OXAs hydrolyze beta-lactam antibiotics differs from the mechanism operating in the classes A and C. In the class A, the active-site serine found at position 70 in element 1 (Ser-x-x-Lys) plays the role of nucleophile in acylation, with Glu166 acting as a general base activating Ser70 during acylation and promoting a water molecule for hydrolysis of the acyl-enzyme intermediate during deacylation. In class D enzymes, the serine in the Ser-x-x-Lys motif would also act as nucleophile, but it is the carbamylated form of the lysine residue that would act as general base in both the acylation and deacylation steps of the catalytic mechanism.

Epidemiology of antibiotic resistance and consumption in Europe (Joint symposium arranged with the ARPAC/EARSS/ESAC)

S127 Antibiotic consumption in Europe: first results from ESAC

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Objectives: ESAC, granted by DG/SANCO of the European Commission (project number: 2001/SID/136), is an international network of national surveillance systems, aiming to collect comparable and reliable antibiotic use data. During a 2-year period (November 2001–October 2003), actions will be taken to harmonize the registration of antimicrobial consumption in all European Countries. A data collection system will be developed allowing to produce comprehensive national data on volume of antibiotic consumption, in ambulatory and in hospital care. Standardized national data will be assembled in a European database for subregional comparison of antibiotic use in relation to antibiotic resistance patterns and socioeconomic and general health parameters.

Methods: ESAC started effectively during the European Conference on antibiotic use in Europe in Brussels, November 2001. It was decided to start data collection retrospective for the period 1997–2001, separately for ambulatory care and hospitals, on a quarterly base using the WHO ATC/DDD classification. Until now, 19 out of 31 participating countries were able to deliver complete requested data, seven could deliver part of the data, and three could deliver spring 2003. Only for two countries, no retrospective data will become available in the near future. A register of all antibiotic products in all package forms available in each country will be updated on a regular base and published on the ESAC website. In the future, national registers will form the base for aggregation of the consumption data enabling validation of the quality of the prospective ESAC data delivery system. Prospective data collection will start mid 2003. Additionally, a database will be constructed containing a comprehensive list of publications in the field of antibiotic consumption, information of all ongoing European research projects, and of all European campaigns aiming to influence antibiotic consumption.

Conclusion: Retrospective annual data for the period 1997–2001 are available for most countries, and detailed prospective data for 2002 will be accessible for scientists and health authorities in order to link utilization data to resistance patterns and to assess the impact of intervention strategies.

S128 Quality of antibiotic consumption data and opportunities for benchmarking

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For several decades and with the exception of Nordic countries, very little was known on antibiotic use in Europe (EU). In 2001, Cars et al. (Lancet 2001; 357: 1851–3) used a combination of commercial data on sales from IMS Health and of data from national systems to provide the first open comparison of antibiotic use in primary health care in EU. In 2002, the 'European Surveillance of Antimicrobial Consumption' (ESAC) project funded by DG-SANCO of the EC extended this benchmark to other EU countries. Problems with IMS Health data include difficulty to access the data for public health research, varying sample sizes among countries, and absence of control for parallel export/import within the EU. ESAC data will be made fully accessible for research, and are presently refined to correct for possible underestimating of antibiotic consumption in a few countries where collection is based on reimbursement claims, when (a) some antibiotics are not reimbursed and therefore excluded from data collection, (b) costs of medicines below a certain threshold are not reimbursed and therefore not registered, or (c) copayment level is high and it is possible to buy antibiotics over-the-counter, outside the reimbursement system. For hospitals, data were recently or will soon be reported by ESAC and by 'Antibiotic Resistance Prevention and Control' (ARPAC), ESCMID's initiative funded by DG-Research of the EC, respectively. While ESAC aims at setting up a surveillance system to obtain national consumption figures, ARPAC focuses on getting antibiotic use and other relevant data from individual hospitals. In both projects, the numerator is the number of defined daily doses (DDDs) as defined by the WHO Collaborating Centre for Drug Statistics Methodology. However, concerns were raised about the quality of denominator data, i.e. the number of occupied bed-days or patient-days, and it might prove preferable to use the number of inhabitant-days to report national consumption figures. For individual hospitals, stratification by hospitalization unit seems essential as an attempt to control for patient case-mix, and provide data useful for benchmarking. In conclusion, additional efforts are needed to improve the comparability of antibiotic consumption data, both in primary health care and in hospitals. These efforts are needed if we want to make sense of the differences in level and patterns of use observed among European countries.

S129 Consumption of antibiotics and resistance to *Streptococcus pneumoniae*: results of an EARSS/ESAC pilot study

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Objective: European Antimicrobial Resistance Surveillance System (EARSS) and European Surveillance of Antibiotic Consumption (ESAC) explored the linkage of their databases investigating the relationship between antibiotic consumption and the resistance to *Streptococcus pneumoniae* (SPN) in Belgium and the Netherlands.
Materials: Penicillin susceptibility testing data on the first isolate of every patient with SPN infection was extracted from the EARSS database for the period 1999–2001. Total antibiotic consumption in ambulatory care in the period 1997–2001 according to ATC/DDD classification was obtained from the ESAC database.
Results: A total of 3004 invasive isolates from Belgium and 2215 from the Netherlands was reported to EARSS in the period 1999–2001. A remarkable higher proportion of penicillin nonsusceptible *S. pneumoniae* (PNSP) was found in Belgium (14%) compared to the Netherlands (1%). Accordingly, total antibiotic consumption in ambulatory care was almost three times higher in Belgium (30, 29, and 27 DDD/1000 inhabitants/day (DID) for 1999, 2000, and 2001, respectively) compared to the Netherlands (10 DID for all 3 years). Proportional use of penicillins was comparable in both countries, small spectrum penicillins were relatively used more in the Netherlands. Per smaller geographical area (province), no relationship was found between antibiotic consumption and resistance patterns within each country. Seasonal fluctuations, with highest antibiotic consumption during winter periods, were observed in both countries and were most pronounced in Belgium. Although a comparable fluctuation in the number of invasive isolates was found, no clear seasonal fluctuations in the proportion of PNSP could be observed. For consumption as well as for resistance, no clear trend over time was observed. Consequently, time series analysis for further exploration of the relationship between consumption and resistance could not be used.
Conclusions: The present pilot study indicates a strong relation between the proportion of PNSP and the amount of antibiotics used in ambulatory care in Belgium and the Netherlands. No relationship between antibiotic consumption and resistance over time could be observed. This pilot study confirmed that the linkage between both databases is feasible, enabling a more in-depth investigation of the intriguing relationship between consumption and resistance in the future.

S130 Inventory of antibiotic resistance and use patterns in European hospitals: first results from ARPAC

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ARPAC is a concerted action project funded by the Research Directorate General of the EC. ARPAC stands for antibiotic resistance, prevention and control. Its objectives are to lay the foundations for a better understanding of the emergence and epidemiology of antibiotic resistance, and to evaluate and harmonize strategies for prevention and control of antibiotic resistant pathogens in European hospitals. Specific aims are to identify antibiotic polices and prescription patterns associated with low resistance rates and to identify infection control policies associated with lower incidence rates of transmissible antibiotic-resistant strains. The study is being conducted by four ESCMID study groups: ESGAP, ESGARS, ESGNI, and ESGEM. The project website is at http://www.abdn.ac.uk/arpac/index. At the beginning of 2003, the number of participating hospitals stood at 267, although hospitals are still actively being encouraged to join the project. Fourteen out of 16 European Union countries are represented and 20 out of 22 other European countries are represented. Initial results on methods of antibiotic susceptibility testing, prevalence of nine alert organisms and antibiotic use during 2001 will be presented. The alert organisms include methicillin-resistant *S. aureus* (MRSA), vancomycin *Enterococci* (VRE), carbapenem-resistant *A. baumannii*, quinolone-resistant *E. coli*, *K. pneumoniae* resistant to the third generation cephalosporins, and *P. aeruginosa* resistant to the carbapenems, aminoglycosides, quinolones, ceftazidime, and cefepime. Data are being collected for both the whole hospital and for the hospitals' ICUs (excluding coronary care units). Data on use of all antibiotics during 2001 are also being collected for the whole hospital and ICUs. Antibiotic use data is being collected using the ABC-calc spreadsheet designed for the purpose at the Statens Serum Institute by D. Monnet. Antibiotic use data will be measured in defined daily doses per 1000 patient days. Data on the patterns of antibiotic resistance and use across Europe will be presented.

Emerging issues in febrile neutropenia patients (Joint symposium arranged with EORTC/IFIG/IATG)

S131 When to add a glycopeptide in an era of multiresistance?

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The empiric initiation of broad-spectrum antibiotics at the onset of fever in granulocytopenic cancer patients (GCP) has been considered a standard therapy for more than two decades. In the 1960–70s, empirical antibiotic regimens were designed for optimal coverage of Gram-negative infections, which were predominant. However, since the mid 1980s, infections caused by Gram-positive (G^+) bacteria, especially coagulase-negative staphylococci and viridans streptococci, have increased in frequency. As a result, considerable controversy has been generated as to the need for empirical specific anti-G^+ therapy at the onset of treatment of fever in GCP. A previous trial of the

IATG-EORTC and of the NCI of Canada (JID 1991; 163: 951) did not support the empiric use of vancomycin given at fever onset in GCP with G^+ bacteremia. A retrospective review of G^+ infections in 550 episodes of febrile neutropenia (Ann. Int. Med. 1988; 108: 30) showed similar results. However, inclusion of a glycopeptide in initial empirical therapy may be justified for selected GCP with conditions such as shock, strong suspicion of catheter-related infection, or known colonization with G^+ bacteria resistant to beta-lactame antibiotics. Frequent changes of antibiotics are a common practice in GCP with persistent fever despite the absence of clinical deterioration and/or the documentation of a microorganism resistant to the allocated regimen. In three large trials (AAC 1996; 40: 1108; Ann. Int. Med 1994; 120: 834; CID 2001; 32: 381), the most frequent treatment modification was the addition of a glycopeptide, mainly on the grounds of persistent fever after 3–4 days of empiric therapy. In a recent double-blind trial, we assessed whether the addition of vancomycin would reduce the time to defervescence in GCP with persistent fever within 48–60 h after the start of empiric monotherapy. Of 763 eligible febrile GCP, 165 with persistent fever were randomized to receive either vancomycin or placebo. The difference in the median time to defervescence was not statistically significant. Moreover, the number of GCP who defervesce, of subsequent infections with G^+ bacteria and the rate of addition of amphotericin B, were also similar in both groups. This study suggests that the empiric addition of vancomycin is of no major benefit in persistently febrile GCP.

S133 Further infections: factors relating the frequency and outcome

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The successful control of initial infectious episode of neutropenia and fever may be followed by emergence of secondary febrile episodes, especially in those patients with various risk factors. In all trials conducted by EORTC-IATG, further infections (or interchangeably named 'superinfections') are defined as 'any episode of fever and/or infection in a neutropenic patient which is not present at initial evaluation, and developed either during antibiotic therapy or within 1 week after discontinuation'. Several risk factors for the development of these infections were previously described. A recent analysis of two large trials performed by EORTC-IATG between 1991 and 1994 involved 1508 patients. Of these patients, 836 responded to the initial empirical treatment. Further infections developed in 129 cases (15.4%). Less than 5% of the patients developed a further infection before day 4. The median time to develop a superinfection was 10 days. Multivariate analysis at the time of the randomization in the trial indicated that age above 16 years and presence of an IV line were predictive for superinfections. A diagnosis of an underlying disease other than acute leukemia was 'protective'. Presence of a clinically documented infection might be associated with a lower rate of superinfections. A further analysis including variables assessed at day 4 after initiation of empirical therapy revealed that in addition to above-mentioned factors stable or increasing granulocyte counts and a granulocyte count of $>100/mm^3$ at day 4 were both independently 'protective' against further infections. Mortality was statistically significant in those patients with superinfections than those without having it (5.4% vs. 1.4%, respectively; $P < 0.01$). Overall, these data underline the significance of superinfections in high-risk febrile neutropenic patients, and may allow to differentiate those who are under risk of developing such infections.

S134 Persistently febrile patients: what to do when everything fails

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Genoa, I

Two therapeutic strategies have been advocated in persistently febrile and neutropenic patients not responding to the initial empiric antibacterial therapy. Empiric antifungal therapy. The possible efficacy of this approach came from two open-label studies performed on both sides of the Atlantic on small patient populations. Pizzo and coworkers randomized 50 patients who were still febrile and granulocytopenic after 7 days of empirical antibacterial therapy and were lacking any documentation of infection, to either discontinue all antibiotic treatments (16 patients), to continue the same combination as the initial treatment (16 patients) or to add empirical amphotericin B (18 patients). Combining the results of the two groups without amphotericin B, four patients developed a deep mycosis, one had *Candida esophagitis* and one had severe *Candida mucositis*. Among the 18 patients receiving amphotericin B there was only one severe mycosis (*Pseudallescheria boydii*). The International Antimicrobial Therapy Co-operative Group of the EORTC randomized 132 persistently febrile and granulocytopenic cancer patients not responding to empirical antibacterial therapy and affected with a fever of unknown origin or a clinically documented bacterial infection, to receive empirical amphotericin B or to continue their antibacterial coverage without modification. There was no statistically significant difference between the two groups in terms of resolution of fever. In a subset analysis, no death due to fungal infection occurred among the patients receiving empirical amphotericin B compared to four in the other group ($P = 0.05$) and the number of documented fungal infections was higher in patients not receiving amphotericin B (6 vs. 1; $P = 0.1$). Empiric glycopeptide addition. The utility of empirical glycopeptide addition was analyzed in two placebo-controlled studies. In the first study (Erjavec Z et al. JAC 2000), no difference was found between teicoplanin and placebo among 114 patients receiving imipenem monotherapy. In the second study (Cometta et al. for the EORTC Antimicrobial Therapy Group, ICAAC 2001), no difference was found between vancomycin and placebo among 165 patients receiving empirical therapy with piperacillin–tazobactam. In conclusion, there is little evidence that any predisposed approach can be recommended in the persistently febrile and neutropenic patient. In these patients every treatment modification should be tailored to the specific clinical situation and to local epidemiological patterns. No modification with watchful waiting can be an option for the experienced physician. Hopefully, in the future we will stop treating fevers and start treating infections in neutropenic patients.

Diagnostics on the verge of a revolution (Joint symposium arranged with FEMS)

S135 The emerging brave new world of microbiology

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A revolution is occurring in nucleic acid analysis, bioinformatics, data storage and retrieval, nanotechnology, physics, microelectronics, and polymer, solid state and combinatorial chemistry. These seemingly disparate fields are being combined and applied to the detection, identification, and characterization of pathogens. The once science fiction scenario of taking a drop of blood, urine or saliva and within an hour knowing whether or not a pathogen is present, its type designation and its antimicrobial resistance potential will soon be a reality. The application of mass spectrometry to microbiology (MALDI-ToF and SELDI-ToF) is also set to have a major impact. These developments, particularly with regard to near patient testing, have important implications for the delivery of health care. They will have an impact on primary care, prescribing practice, organization of pathology laboratories, counseling services, surveillance and epidemiology, and for medico-legal practice. (S. P. Borriello [1999] BMJ 319: 298–301). New molecular methodologies are facilitating real-time strain differentiation for out-break investigations with detection of antimicrobial susceptibility potential. Further, new light is being shed on epidemiology of infectious diseases for established diseases, and new pathogens are being uncovered.

S136 Laboratory diagnostics: from eminence based to evidence based

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The overload of medical information and technical possibilities and the increasing need for cost control of medical interventions led to the concept

of evidence-based medicine (EBM). Evidence-based diagnostic microbiology is an integral part of EBM. Diagnostic tests should thus be scrutinized for the evidence on which they are based. The criteria have been clearly summarized by Sackett et al. (2000) to which one criterion should be added: the evaluation should be done on a sufficiently large number of specimens of patients to reduce the confidence interval of the result. For each laboratory test the question should be asked how much the information obtained by a test will be beneficial for the patient and at what cost. Criteria to be applied to the comparison between tests have been better defined, particularly when a new test is more sensitive than the previously used standard test: these will be discussed. Exclusion criteria for several categories of specimens, e.g. urine and stool specimens submitted to the laboratory have been proposed and are largely implemented. Exclusion criteria for other specimens such as sputum, herpes simplex detection in CSV or urine testing for *Mycobacterium tuberculosis* could also be proposed and will be discussed. In other instances tests could/should be limited to patients with particular risk factors such as in screening tests for *Chlamydia trachomatis* or human papilloma virus. One of the most difficult area is that of the respiratory tract infections. Factors related to the specimen, to the detection methods and to the patient are responsible for the important uncertainty of many laboratory results. As a result of the low sensitivity and specificity of sputum examinations, efforts should be reoriented and resources should be reinvested in alternative techniques to improve significantly the diagnosis and management of respiratory infections. For many applications nucleic acid amplification tests resulted in important diagnostic progress. For each of these tests, their contribution in routine diagnosis should be validated taking into account not only the validity of the test but also the prevalence of the disease. Finally, a cost benefit analysis should reveal the opportunity for the introduction of diagnostic tests. In conclusion, much evaluation exercises and thinking remains to be done for EBM. This can only be realized in close collaboration between clinicians and microbiologists.

S137 Real-time PCR – why and where not

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As compared to 'conventional PCR' which measures the concentration of a specific product (the amplicon) at the end of the amplification process, 'real-time PCR' refers to the possibility of continuously monitoring the increasing amount of accumulated product. Even though this monitoring is not really continuous, it allows to follow the kinetics of the reaction and thus provides a new approach to quantitation. The term 'homogenous' PCR which is often used instead of 'real-time PCR' indicates that amplification and detection of the amplicon ore done in the same closed reaction vessel eliminating the need for further amplicon analysis by gel electrophoresis, Southern blotting, microtiter plate hybridization, etc. The advantages of real-time PCR are: (1) reduced susceptibility to carry-over contamination, (2) shorter turnaround time (depending on the platform used), (3) less hands-on time, and (4) the potential for easier quantitation over a greater range (5–6 logs) than conventional PCR (2–4 logs). Whereas the first three aspects are similarly valid for most diagnostic applications, quantitative analysis has significantly improved the clinical usefulness of results in the field of virology only, especially for organisms detected in blood or serum. In contrast, quantitation in bacterial diagnostics may to a large extent be considered inadequate because of varying quality of the specimens analyzed, nonhomogenous distribution of pathogenic organisms in specimens, dependence of PCR-efficiency on total amount of nucleic acids in a specimen, etc. Despite these limitations, clinical diagnostic laboratories may profit to a large extent from these new technological developments which have already resulted in a number of different chemistries used (Fluorescence Resonance Energy Transfer/FRET, 5′ nuclease assay/TaqMan, Molecular beacons, SybrGreen in combination with melting curves) as well as in a variety of different equipment.

New antibiotics and pharmacokinetics

O139 Ertapenem 1 g once a day is highly effective for treatment of community-acquired and mixed infections in adults with diabetes

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Objectives: In clinical trials, ertapenem, a once-a-day parenteral beta-lactam licensed in Europe in April 2002, was highly effective for treatment of complicated intra-abdominal (IAI), acute pelvic (PI), complicated skin–skin structure (SSSI), and complicated urinary tract infections (UTI) and community-acquired pneumonia (CAP). Ertapenem vs. comparator cure rates in these trials were: IAI, 176/203 (87%) vs. 157/193 (81%); PI, 153/163 (94%) vs. 140/153 (92%); SSSI, 152/185 (82%) vs. 147/174 (84%); UTI, 229/256 (89%) vs. 204/224 (91%); CAP, 335/364 (92%) vs. 270/294 (92%). Such infections in patients with diabetes often are more difficult to treat. Objectives of this post hoc analysis were to examine demographic/disease characteristics of patients with and without diabetes, and compare the efficacy of ertapenem in diabetic patients with piperacillin–tazobactam (PT) and ceftriaxone (CRO).
Methods: Seven randomized, double-blind comparative trials assessing the efficacy of ertapenem, 1 g once a day, were conducted worldwide. Comparator agents, considered standard of care, were PT 3.375 g Q6H (IAI, PI, SSSI) and CRO 1 g once a day (CAP, UTI). Clinical and/or microbiologic efficacy was assessed in evaluable patients at prespecified timepoints post-therapy, defined by indication.
Results: Of the 3255 patients treated in seven trials, 493 (15%) had diabetes. In all studies except PI, diabetics were older than those without diabetes. Compared to patients without diabetes, in IAI, CAP, and UTI studies, respectively, a lower proportion of diabetics had appendicitis, Pneumonia Severity Index 'T3, and acute pyelonephritis'. Duration of therapy was similar in those with and without diabetes in all studies. Among diabetics, treatment groups were generally similar with respect to demographic, disease, and treatment characteristics. Cure rates (%) (clinical and microbiologic for IAI; clinical for PI, SSSI, CAP; microbiologic for UTI) for evaluable patients with diabetes are shown in the Table 1.

	IAI	PI	SSSI	Overall	CAP	UTI	Overall
Ertapenem	5/6 (83)	9/9 (100)	41/56 (73)	55/71 (77)	38/40 (95)	34/43 (79)	72/83 (87)
PT	16/18 (89)	8/9 (89)	36/47 (77)	60/74 (81)	–	–	–
CRO	–	–	–	–	35/39 (90)	35/43 (81)	70/82 (85)

Conclusions: In the subgroup of patients with diabetes, ertapenem was highly effective for treatment of SSSI, CAP, and UTI. Ertapenem also was efficacious in diabetics with IAI and PI, but the numbers of patients were small. Cure rates for SSSI and UTI were lower in diabetics than patients without diabetes, but were similar for ertapenem and PT or CRO.

O140 In vitro activity of Oritavancin and comparator agents against *Streptococcus pneumoniae* collected from European Laboratories during 2000–2001

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Objectives: *S. pneumoniae* (SP) is a common causative agent of both community-acquired and hospital respiratory tract infections worldwide. The increasing prevalence of antimicrobial resistance among SP, including multidrug resistance (MDR), threatens the use of empiric therapies and creates treatment challenges for physicians. Oritavancin (ORI) is a novel semisynthetic glycopeptide that has shown in vitro activity against SP, including isolates that are resistant to commonly prescribed antimicrobials.
Methods: We examined the activity of ORI and comparator agents against 561 SP collected from patient specimens at hospital laboratories in 15 European countries during 2000–2001. Isolates were selected to include both susceptible (S) and resistant (R) phenotypes. Isolates were centrally tested by NCCLS broth microdilution, and MICs were interpreted using the 2002 NCCLS published breakpoints.

Results: Based on MIC90, ORI (0.002 mg/L) activity was superior to vancomycin (VAN; 0.25 mg/L), linezolid (1 mg/L), and quinupristin–dalfopristin (0.5 mg/L). ORI showed consistent activity against penicillin (PEN) S ($n = 368$), PEN I ($n = 117$) and PEN R ($n = 76$) isolates with MIC90s of 0.002, 0.002, and 0.004 mg/L, respectively. Similarly, ORI showed consistent activity against non-MDR ($n = 532$; ORI MIC90, 0.002 mg/L) and MDR isolates ($n = 29$; ORI MIC90, 0.004 mg/L), including those that were resistant to four antimicrobial classes (PEN, erythromycin, levofloxacin, trimethoprim–sulfamethoxazole). In comparison, VAN showed an MIC90 of 0.25 mg/L against both non-MDR and MDR SP.
Conclusions: ORI demonstrated superior in vitro activity against S and R SP, with MIC90s five to nine doubling dilutions lower than VAN and the comparator agents tested. ORI is currently in Phase III clinical trials for the treatment of Gram-positive infections.

O141 In vitro activity of daptomycin against vancomycin-resistant enterococci, linezolid-resistant vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* and penicillin-resistant pneumococci

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Objectives: The in vitro activity of daptomycin was studied against clinical isolates of vancomycin-resistant enterococci (VRE), linezolid-resistant VRE (LRVRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and penicillin-resistant (MIC ≥ 2 μg/mL) *Streptococcus pneumoniae* (pRSp).
Methods: Vancomycin resistance genotype was determined by PCR amplification with primers specific for the vancomycin resistance associated genes *vanA*, *vanB*, *vanC-1* and *vanC-2/3*. Daptomycin MIC values were determined using broth microdilution in 0.1 mL total volume of cation supplemented Mueller–Hinton broth (CAMHB) supplemented to a final calcium concentration of 50 mg/L with an initial inoculum of 10×10^4 cfu per well of logarithmically growing bacteria. Pneumococcal MIC media was supplemented with 3% lysed horse blood. MIC tests were incubated 18 h in room air at 35 °C. Daptomycin MBC values were determined by subculture of the MIC tests. Time-kill studies (expressed as log 10 cfu/mL) were performed in 25 mL CAMHB (50 mg/L calcium) with an initial inoculum of 10^4 to 10^6 cfu/mL and incubated for 24 h at 35 °C, room air. Bactericidal activity was defined as 99.9% killing (≥ 3 log 10 decrease in cfu/mL) of the initial inoculum.
Results: The results of daptomycin susceptibility testing are summarized in the table below as MIC90, MIC range, MBC90, and MBC range (μg/mL). Time-kill studies with 11 LRVRE demonstrated ≥ 3 log 10 cfu/mL reduction of initial inoculum after 24 h with concentrations of 32 μg/mL or less of daptomycin.

Organism	No. Isolates	MIC_{90}	MIC Range	MBC_{90}	MBC Range
vanA VRE	11	4	1–4	16	2–16
vanB VRE	11	4	0.5–4	16	2–32
vanC1 VRE	7	–	1–2	–	8–32
vanC2/3 VRE	8	–	<0.125–0.25	–	4–8
vanA LRVRE	11	4	0.5–8	16	1–16
MRSA	33	0.5	≤0.125–1	4	0.25–8
pRSp	25	≤0.125	All ≤ 0.125	≤0.125	≤0.125–0.25

Conclusions: Daptomycin is active in vitro against VRE, LRVRE, MRSA and pRSp. Daptomycin showed bactericidal activity in time-kill studies against LRVRE.

O142 Iclaprim, a novel broad-spectrum antibiotic

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In recent years, there has been an alarming increase in microbial resistance to current clinical therapies. For example, up to 50% of staphylococci are resistant to methicillin (MRS) and resistance to vancomycin is increasing (VISA/GISA). Importantly, it had been predicted that staphylococci could exhibit high-level resistance to vancomycin and at least two cases of such resistance have recently been reported (VRSA). If such resistance spreads then only few life-saving alternatives will remain for the clinicians. Similarly, increasing resistance to many drugs by streptococci and other respiratory tract pathogens is making therapy of such infections (RTI) more and more difficult. Iclaprim (formerly AR-100) is a new compound that was designed to have expanded activity against Gram-positive and Gram-negative organisms by specifically and selectively inhibiting bacterial dihydrofolate reductase. This synthetic diaminopyrimidine derivative exhibits broad-spectrum activity against major pathogens, including multidrug resistant pathogens. Iclaprim is particularly active against MRS, VISA/GISA, macrolide and quinolone-resistant strains. The compound also exhibits good activity against respiratory tract pathogens such as multidrug resistant streptococci and *H. influenzae*. Preclinical and Phase I studies suggest that Iclaprim could prove to be a useful agent for severe hospital infections as well as RTI. The compound is currently in Phase II clinical trials.

O143 Dalbavancin: phase 2 demonstration of efficacy of a novel, weekly dosing regimen in skin and soft tissue infections

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Objectives: Dalbavancin (DAL) is a novel semisynthetic glycopeptide in phase 3 development, more active in vitro and in animal models than vancomycin or teicoplanin. The PK profile and in vitro and in vivo activity of DAL against Gram-positive bacteria, including most drug resistant strains, predict that a single dose will provide therapeutic concentrations for at least one week. The objectives of this study were to compare the safety and efficacy of two DAL regimens to investigator-specified standard of care (comparator) in patients with skin and soft tissue infections (SSTI).
Methods: This phase 2 randomized, controlled study was conducted in adult patients with SSTI involving deep skin structures or requiring surgical intervention. Patients were randomized to one of three arms: DAL 1100 mg i.v. single dose; DAL 1000 mg i.v. day 1 and 500 mg i.v. on day 8, or comparator for 7–21 days. Adverse events and labs were assessed. Blood samples for PK were collected from patients on DAL. The primary endpoint was clinical response at follow up (FU) in clinically evaluable patients.
Results: Sixty-two pts were enrolled. The majority (89%) of SSTI were classified as deep infection. The most common comparators were clindamycin, ceftriaxone, vancomycin and cefazolin. Mean comparator group treatment duration was 15 days. The most common pathogen was *S. aureus* (38% MRSA). Outcomes in the evaluable populations were as follows:

Endpoint	DAL 1 Dose $n = 20$	DAL 2 Doses $n = 21$	Comparator $n = 21$
Clinical success at FU (%, *n*/*N*)	61.5 (8/13)	94.1 (16/17)	76.2 (16/21)
Micro success at FU (%, *n*/*N*)	27.3 (3/11)	72.7 (8/11)	64.3 (9/14)

DAL 2 doses resulted in numerically higher response rates than DAL 1 dose or comparator. There was no difference in outcomes for MRSA vs. MSSA. Both regimens maintained plasma concentrations well above the MIC90 of the *S. aureus* isolates (0.12 mg/L) through at least a week following the last dose. DAL was well tolerated. AEs were infrequent and similar across study arms. There were no trends in any lab abnormalities in DAL treated pts.
Conclusion: This is the first efficacy data for a new glycopeptide with a novel dosing regimen. DAL was well tolerated; two weekly doses were effective in deep SSTI and comparable to standard of care. DAL is currently under study in phase 3 clinical trials for SSTI.

O144 A randomized, double-blind, placebo- and positive-controlled study of the effects of a new antibacterial, TD-6424 on cardiac repolarization (the QTc interval) in healthy subjects

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Background: TD-6424 (TD) is a new antibiotic that exerts rapid, concentration-dependent bactericidal activity against clinically important Gram-

positive bacteria. Unlike other glycopeptides, TD inhibits bacterial lipid synthesis in addition to peptidoglycan synthesis. Clinical doses are expected to be 7.5–15 mg/kg once daily. Preclinical and early clinical data suggested a possible effect of TD on prolonging the QTc interval in this dosage range.
Objectives: To determine the effects of TD on the QTc interval in healthy human subjects.
Methods: 160 subjects (94 M, 66 F) were randomized into 4 groups to receive TD at a dose of 7.5 mg/kg or 15 mg/kg, placebo (TD vehicle) or moxifloxacin 400 mg (positive control). Moxifloxacin is widely used, and is known to prolong the QTc interval by approximately 9 ms following i.v. administration. All medications were administered once daily for 3 days as 60 min i.v. infusions. Sixteen ECGs were obtained over 24 h following an infusion of D5W (baseline) and following the Day 3 infusions of each medication. ECG's were analyzed digitally in a blinded fashion by a core lab. QT data were corrected for heart rate by Fridericia's method (QTcF), and are displayed as mean and maximum (max) change from baseline.
Results: Complete data are available from 151 subjects. No subject had a QTcF $\geq$ 450 ms and none experienced clinically significant ECG abnormalities. Preliminary results are displayed in the table.

	Placebo	TD 7.5 mg/kg	TD 15 mg/kg	Moxifloxacin
No. of subjects	39	39	34	39
Day 3 Mean change QTcF	−1.0 ms	2.8 ms	3.8 ms	8.1 ms
≥30 ms	0	0	0	0
Day 3 Max change QTcF	38 ms	46 ms	63 ms	55 ms
30 to <60 ms	5 (13%)	9 (23%)	6 (18%)	15 (38%)
≥60 ms	0	0	1 (3%)	0

Mean change in QTcF for moxifloxacin, serving as the assay sensitivity positive control, was significantly greater than the other groups, and all active treatment groups were greater than placebo. The TD groups were not significantly different from each other. Placebo-corrected mean change in QTcF values for TD 7.5 mg/kg, 15 mg/kg and moxifloxacin are 3.8 ms, 4.8 ms, and 9.1 ms, respectively.
Conclusions: These data demonstrate minimal (<5 ms) prolongation of the QTc interval by TD, even at the highest dose that is likely to be used clinically. In this study, the change in QTc for TD was less than that observed for moxifloxacin.

O145 Efficacy and tolerability of long-term pleconaril chemoprophylaxis for picornavirus illness in adults

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Background: The investigational orally administered, capsid-binding, anti-picornavirus drug pleconaril has shown to be effective for treatment of picornavirus colds in adults.
Objectives: To assess the tolerability and efficacy of long-term pleconaril in preventing picornavirus morbidity.
Methods: We conducted a randomized, double-blind, placebo (P)-controlled study during August–October 2001; 1069 healthy adults were enrolled at 13 centers and randomized in a 1 : 1 : 1 ratio to pleconaril 400 mg QD or BID or P for 6 weeks. The main efficacy outcome was laboratory proven, self-diagnosed picornavirus colds.
Results: Documented picornavirus illnesses were observed in 20% of P, 13% of pleconaril QD, and 12% of pleconaril BID recipients ($P < 0.01$, pleconaril QD or BID vs. P). Picornavirus-associated missed school or work (32–36% reductions), functional impairment (38–43%) and sleep disturbance (38–43%) were all significantly reduced in pleconaril recipients compared to P. Discontinuations due to possible adverse events were observed in 3% P, 4% pleconaril QD, and 6% pleconaril BID. Headache, nausea, and diarrhea were among the most frequently reported adverse events in all treatment groups. Among women taking oral contraceptives, menstrual disorders were reported two to three times more frequently in the pleconaril groups compared to P.
Conclusions: Pleconaril is the first oral antiviral chemoprophylactic agent proven to reduce picornavirus morbidity in adults. Pleconaril was generally well tolerated when used for prolonged dosing, although it showed evidence of drug interactions with oral contraceptives.

O146 Pharmacokinetic interactions of Ceftazidime and Amikacin in healthy volunteers

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Objectives: In vitro findings on the synergy between ceftazidime and amikacin on multidrug resistant isolates (Giamarellos-Bourboulis et al. DMID 1997; 29: 81) create the necessity to study any probable pharmacokinetic interaction between them.
Methods: Antimicrobials were administered single and in combination in six healthy volunteers at 15-day intervals. One gram of ceftazidime was administered intravenously within 30 min followed or not by the bolus infusion of 0.5 g of amikacin. Blood and urine samples were collected at regular time intervals and drug serum levels were estimated by a microbiological assay. The indicator strain applied for the estimation of the levels of ceftazidime became resistant to amikacin after serial exposure to sequentially increased concentrations. The strain applied for the estimation of the levels of amikacin became resistant to ceftazidime by a similar method.
Results: Mean C_{max}, AUC, serum half-life, clearance and percentage 8-h urine excretion of ceftazidime when administered single were 26.50 mg/L, 69.76 mg/h.l, 01.59 h, 286.10 mL/min and 57.99%, respectively. They were changed to 25.55 mg/L (pNS), 82.03 mg/h.l (pNS), 01.70 h (pNS), 208.51 mL/min (pNS) and 35.40% (pNS), respectively, after co-administration with amikacin. Respective values for amikacin when administered single were 13.04 mg/L, 38.43 mg/h.l, 02.80 h, 293.30 mL/min and 71.75%. They were changed to 19.59 mg/L ($P = 0.007$), 60.35 mg/h.l ($P = 0.05$), 02.51 h (pNS), 141.03 mL/min (pNS) and 82.19% (pNS), respectively, after coadministration with ceftazidime.
Conclusions: The co-administration of ceftazidime and amikacin does not affect pharmacokinetics of ceftazidime. It does, however, increase the distribution of amikacin as defined by increases of Cmax and AUC without affecting its rate of elimination. The described increases of distribution might be helpful for the eradication of infections by multidrug resistant isolates when both agents are prescribed.

O147 Concentrations of piperacillin and tazobactam in bone of different origin

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Objectives: The concentrations of piperacillin and tazobactam in bone have been studied for hip bone of mostly cancelous structure. Jaw bone is known to have different histologic and mechanical characteristics.
Methods: The penetration dynamics of piperacillin (PIP)-tazobactam (TAZ) into facial and hip bone tissues were investigated in 10 healthy patients undergoing surgical bone resections for orthognatic reasons. All patients had similar age, body weight and renal clearance. The drug was administered at the start of surgery in a single dose [4 (PIP) + 0.5 g (TAZ) i.v.]. Depedning on the surgical procedure 1–4.5 h later bone and plasma samples were drawn. A mass spectroscopy technique LC-MS/MS was used to study the drug concentrations.
Results: The concentration ratios of PIP-TAZ were 4.3–10.2 in facial bone ($n = 6$), 7.6–7.9 in hip bone ($n = 2$) and 5.8–9.3 in plasma. The mean ratios of drug concentrations in bone and plasma for facial and hip bone were 5–45 and 3–20%, respectively, for PIP and 4–36 and 4–16%, respectively, for TAZ.
Conclusions: The bone/plasma ratio seemed to be higher for facial bone however, a large range should be taken into account. The PIP/TAZ ratio was similar in facial and hip bone and in plasma. According to earlier studies this indicates sufficient antibeta-lactamase activity of TAZ in facial bone.

O148 Pharmacokinetics of sulbactam and piperacillin in critically ill patients during continuous veno-venous hemodiafiltration

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Objective: Continuous extracorporeal treatment of acute renal failure is frequently used in critically ill patients. Likewise, patients presenting with

sepsis and multiple organ failure are often treated with piperacillin and beta-lactamase inhibitors. Although frequently administered, pharmacokinetic data of sulbactam during continuous veno-venous hemodiafiltration (CVVHD) are scarce and inconclusive. We, therefore, investigated the pharmacokinetics of sulbactam in combination with piperacillin in critically ill patients with anuric renal failure reliant upon CVVHDF.
Methods: Single dose pharmacokinetics were studied in nine patients after short-time infusion of 1 g sulbactam in combination with 1, 2 or 4 g piperacillin. In addition, multiple dose pharmacokinetics were investigated at steady-state in six patients receiving 1 g sulbactam b.i.d. or t.i.d. in combination with piperacillin 2 g b.i.d., 4 g b.i.d. or 4 g t.i.d. Pharmacokinetic parameters were determined from repeated blood and dialysate sampling up to 12 h after sulbactam dosing.
Results: Sulbactam pharmacokinetics – for the single dose, total clearance (Cl) was 84 ± 37 mL/min, Cl by CVVHD (ClCVVHDF) was 24 ± 10 mL/min and elimination half-life (t1/2) 5.9 ± 1.5 h. Multiple dose pharmacokinetics at steady state for the b.i.d. and t.i.d. sulbactam dosing schedules were: Cl 71 vs. 37 mL/min, ClCVVHDF 21 vs. 11 mL/min and t1/2 5.2 vs. 10.6 h, respectively. Piperacillin Cl was in the order of 60 mL/min, ClCVVHDF 15 mL/min and t1/2 6 h. There was no pharmacokinetic interaction between sulbactam and piperacillin. Sulbactam and piperacillin clearances were significantly correlated ($r = 0.71$, $P < 0.001$), but not t1/2. The data indicate a higher than expected sulbactam accumulation and a reduction of nonrenal drug elimination during t.i.d. treatment.
Conclusion: Differing from previous recommendations the optimal dosage of sulbactam is proposed to be 1 g b.i.d. in anuric atients reliant upon CVVHDF.

Pathogenetic mechanisms of bacterial infections

O149 Structural characterization of cytokine inducible lipoteichoic acid by use of synthetic derivatives

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Objectives: There is increasing evidence that lipoteichoic acid (LTA) from the cytoplasmic membrane of Gram-positive bacteria is an immunostimulatory counterpart to lipopolysaccharide (LPS). We have addressed LTA of *Staphylococcus aureus* as a foremost Gram-positive pathogen. Chemical synthesis of biologically active LTA based on the structure of LTA from *S. aureus* was carried out. We modified the structure of this synthetic LTA and used a human whole blood cytokine release assay to define the key components of the LTA molecule necessary for inflammatory stimulation. Furthermore, we examined the toll-like receptor (TLR) dependency of the derivatives.
Methods: Blood of healthy donors was stimulated with native LTA of *S. aureus* or synthetic derivatives in a standardized ex vivo whole blood test. Peritoneal cells were isolated from TLR2 deficient and wildtype (wt) mice and incubated with native LTA or derivatives. The cytokine release was determined in the supernatants by ELISA.
Results: The synthetic LTA (D-Ala-LTA) consists of a gentiobiosyl-sn-dimyristoylglycerol anchor joined to six glycerophosphate units carrying four D-alanine and one GlcNAc substituent. Native LTA has 30–50 glycerophosphate units with similar substituents. After stimulation of whole blood with native or synthetic LTA we observed an equal cytokine pattern and potency. Replacing D-alanine by L-alanine attenuated the stimulatory activity indicating stereoselective recognition. LTA anchor alone is less potent in inducing cytokines though it is necessary for biological activity of LTA. Replacement of the GlcNAc by a further D-alanine substituent played no decisive role in the biological activity of LTA. Synthetic LTA without the gentiobiose subunit also had no significant effect on cytokine release. Peritoneal cells with a homozygous TLR2 defect showed no significant cytokine release after stimulation with native LTA or derivatives.
Conclusions: Taken together, the key components of the LTA molecule necessary for stimulation of monocytes are the LTA anchor with two fatty acids and a glycerophosphate backbone with D-alanine substituents.

O150 Polymorphism of TLR2 or 4 does not affect human or murine *Chlamydia pneumoniae* infection

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Objectives: *Chlamydia pneumoniae* has a sero-prevalence of 70% in adults and leads to persistent infections, which have been linked to chronic inflammatory diseases like atherosclerosis. Functionally, defective polymorphisms of toll-like receptors (TLR), crucial for the recognition of bacteria by the innate immune system, have been associated with susceptibility to bacterial infections. Since TLR2 and, at least in part, TLR4 are mandatory for macrophages to respond to *C. pneumoniae*, we investigated the effect of homozygous and heterozygous nonfunctional polymorphisms of TLR2 and 4 on the susceptibility of humans and mice towards *C. pneumoniae*.
Methods: Bone marrow derived macrophages (BM) from wild-type (wt) mice or mice with a homozygous or heterozygous nonfunctional mutation of TLR2 or 4 were incubated with 10exp6 *C. pneumoniae* for 24 h and TNF and IL-6 release was detected by ELISA. Wt mice and mice with a homozygous defect in TLR2 or 4 were infected intranasally with 10exp6 *C. pneumoniae* and the bacterial burden of lung and bronchoalveolar fluid (BAL) was determined at day 18 after infection by a highly sensitive and specific real-time PCR. Anti-*C. pneumoniae* IgG was determined by microimmunofluorescence test (MIF) adapted for mice. Blood of 160 healthy donors was genotyped for the Arg753Gln and for the Asp299Gly polymorphism of TLR2 and TLR4, respectively, anti-*C. pneumoniae* IgG and IgA were analyzed by MIF and whole blood cytokine response was determined.
Results: BM derived from wt mice or mice with a homozygous or heterozygous mutation of TLR2 or 4 responded similarly towards stimulation with *C. pneumoniae* while BM from mice with a homozygous defect in TLR2 showed a significantly decreased release of cytokines. After infection with *C. pneumoniae*, the bacterial load of lung and BAL of wt mice and mice with a homozygous defect in TLR2 or 4 was alike and all groups developed comparable anti-*C. pneumoniae* IgG levels. Of the 160 donors 6.3% were found to be heterozygous for the TLR2 and 9.4% heterozygous for TLR4 polymorphism, prevalence of anti-*C. pneumoniae* IgG and IgA was similar in all groups. No apparent differences in cytokine response to *C. pneumoniae* were found.
Conclusions: Taken together, these data indicate that in vivo neither a functional TLR2 nor TLR4 is necessary for effective clearance of *C. pneumoniae* in murine infection, but at least a functional TLR2 allele is necessary for adequate cytokine response. For humans, no dependence of anti-*C. pneumoniae* sero-prevalence on heterozygous TLR polymorphisms was observed.

O151 Heterozygous dysfunctional toll-like receptor 2 and 4 polymorphisms do not affect the cytokine response of blood leukocytes

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Objectives: Functionally defective heterozygous polymorphisms of the toll-like receptor (TLR)-2, which is crucial for the recognition of Gram-positive bacteria and TLR4, the key receptor for Gram-negative bacteria, have recently been associated with predisposition to infectious diseases and atherosclerosis, which was attributed to changes in inflammatory responses. To test this hypothesis, we investigated the effect of nonfunctional heterozygous TLR2 and 4 polymorphisms on bacteria-induced cytokine release in humans and mice.
Methods: Blood from 160 healthy donors was genotyped for the Arg753Gln and the Asp299Gly polymorphism of TLR2 and 4, respectively, and the cytokine release inducible by Gram-positive or Gram-negative bacteria and bacterial cell wall components was determined in a standardized ex vivo whole blood test. Bone marrow-derived macrophages (BM) from wild-type (wt) mice or mice with a homozygous or heterozygous nonfunctional mutation of TLR2 or 4 were incubated with different bacterial stimuli and TNF and IL-6 release were determined by ELISA.
Results: Nine per cent of the 160 donors were heterozygous and 0.6% homozygous carriers of the TLR4 polymorphism. Their cytokine release in response to 1 μg LPS/mL did not differ significantly from the wild-type

carriers for any cytokine (TNFa, IL-1b, IL-6, IFNg, G-CSF) or eicosanoid (prostaglandin E2, thromboxane, leukotriene B4) measured except for IL-10. The same held true for serum cytokines and C-reactive protein. To exclude that differences become evident only at lower stimulus concentrations or in response to live pathogens, 10 subjects with heterozygous polymorphism and 12 wild-type controls were recruited for a follow-up study. They responded alike, except for IL-10 formation, to a series of concentrations of LPS and live *E. coli*. A total of 6.3% of the 160 donors were heterozygous carriers of the TLR2 polymorphism and six of them could be recruited again for ex vivo stimulations with serial dilutions of lipoteichoic acid and whole *S. aureus*. However, their response was not different from wild-type controls for any endpoint. The bacteria-induced cytokine release of BM derived from mice with heterozygous polymorphisms of TLR2 or 4 was comparable to wt mice, but significantly reduced for BM derived from mice with a homozygous mutation.

Conclusion: These data argue against an alteration of inflammatory responses towards pathogens conferred by a heterozygous polymorphism of TLR2 or 4.

O152 Apoptosis-resistant profile of neutrophil granulocytes during acute exacerbation of chronic bronchitis

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Background: The pathogenesis of acute exacerbations of chronic bronchitis (AECB) is not fully understood. A responsible pathogen is usually isolated in only 50–80% of the AECB. A major feature of AECB appears to be the massive invasion of activated neutrophils into the bronchial tree. Basically, this neutrophil infiltration is the result of the chemotactic activity of cytokines. In addition, there is evidence that apoptosis resistance of these cells could also increase the total granulocyte count.

Methods: Twenty-two hospitalized patients with AECB were included. Three sequential heparin blood samples were obtained at admission, after 3–5 days and before discharge. Apoptosis was induced by incubating the isolated granulocytes at 37 °C and 5% CO_2 for 18 h. Apoptotic rate before and after induction was measured by flow-cytometry (Annexin V/7 AAD staining) and by cell microscopy (chromatin condensation). Apoptosis rates during AECB were contrasted using Wilcoxon test. The correlation between the two methods of measurement was evaluated by Pearson's coefficient.

Results: Apoptosis resistance of granulocytes was decreasing during the AECB. The mean percentages of apoptotic granulocytes were 36.5% on admission day, 42% after 3–5 days and 51% at discharge. There was a statistically significant difference between apoptosis rates on the first day and at discharge ($P = 0.034$). The methods used for measuring apoptosis showed a high level of correlation ($r = 0.745$, $P < 0.01$).

Conclusion: During acute exacerbations of chronic bronchitis, neutrophil granulocytes show an apoptotic resistant behavior that is diminishing progressively after treatment and clinical remission.

O153 GM-CSF limits the intracellular growth of *Listeria monocytogenes* in THP-1 macrophages

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Objectives: *Listeria monocytogenes* is a life-threatening pathogen that affects mainly immunosuppressed patients. GM-CSF, a cytokine that induce macrophages, monocytes and polymorphonuclears production, differentiation and activation, is clinically used as immunostimulator after chemotherapy and in immunocompromized patients. It has been shown that mice knocked out for the gene that codes for GM-CSF are more susceptible to *L. monocytogenes* infection and are dying more rapidly. We have thus investigated whether GM-CSF would have a direct influence on the intracellular growth of *L. monocytogenes* in a model of THP-1 human macrophages.

Methods: GM-CSF receptors were quantified on THP-1 cells by measuring the specific binding of [125I]-GM-CSF. To assay the effect of GM-CSF, THP-1 macrophages were preincubated with several concentrations of GM-CSF (respectively 5, 10, 20, and 40 ng/mL) during 24 and 48 h, washed and infected with *L. monocytogenes* (AAC, 46 : 2095–2103). The number of viable bacteria (CFU) was determined after 5 h of incubation. We compared these results to the effect of IFN-gamma (a cytokine known to strongly limit intracellular *L. monocytogenes* growth).

Results: THP-1 macrophages display high affinity GM-CSF receptors (KD = 13.87 pM, number of receptors per cell: ~100). GM-CSF decreases the number of CFU in a concentration-dependent manner when above a threshold concentration. Indeed, at 5 ng/mL, there was no difference with the control, at 10 ng/mL there was $9.2 \pm 0.8\%$ of growth reduction and reached a maximal effect at 20 ng/mL with 19.9 ± 1.8 (no further reduction was obtained with 40 ng/mL). There was no difference between 24 and 48 h of preincubation. After 24 h of preincubation at 100 U/mL, IFN-gamma reduced the growth of $40.9 \pm 0.5\%$. To confirm that the effect was mediated by the GM-CSF itself, cells were preincubated with 20 ng/mL of GM-CSF and 2.5 µg/mL neutralizing monoclonal antibodies against the GM-CSFR-alpha subunit, and the effect of GM-CSF was then abolished.

Conclusion: GM-CSF limits the intracellular growth of Listeria monocytogenes inside THP-1 human macrophages but is less potent than IFN-gamma.

O154 Increased neurogenesis after experimental *Streptococcus pneumoniae* meningitis

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Objectives: Proliferation of neural progenitor cells is increased in response to several modes of brain injury. In this study, proliferation of progenitor cells after experimental *S. pneumoniae* meningitis and expression of neuronal marker proteins suggesting differentiation into mature neurons was investigated.

Methods: Male C57BL/6 mice ($n = 35$) were infected by injection of log 4 CFU of *S. pneumoniae* into the right forebrain. Controls ($n = 30$) received an injection of saline. 24 h later antibiotic therapy was initiated with ceftriaxone 100 mg/kg twice daily over 5 days. On day 2, 6, 10 and 16 after infection four respective groups of mice (six infected animals, six controls) received five intraperitoneal injections of bromodeoxyuridine (BrdU) 50 mg/kg at 3-h intervals and were sacrificed 18 h later. To assess long-term survival of BrdU-labeled cells, 6 infected animals and 6 controls were treated with BrdU (50 mg/kg) twice daily from day 7 until day 10 after infection and were killed 4 weeks thereafter.

Results: All mice infected with *S. pneumoniae* developed meningitis. 5 mice died during the acute phase of meningitis and were excluded from the analysis. The other animals fully recovered from the infection. Repeated tight-rope tests revealed a higher impairment of physical activity in meningitic mice compared to noninfected controls ($P = 0.001$). At 6 days after infection, the density of BrdU-labeled progenitor cells in the dentate gyrus of the hippocampal formation was higher in the meningitis group than in controls, [median (25th/75th percentile): 22.2/mm^2 (16.7/26.4) vs. 8.7/mm^2 (5.8/9.5); $P = 0.004$]. At 2 and 10 days after infection differences almost reached statistical significance ($P = 0.06$). Survival of newborn cells 4 weeks after the last BrdU injection was higher after meningitis than in controls as indicated by double-labeling for BrdU and the neuronal marker MAP-2 [33.8/mm^2 (3/80.8) vs. 0.7/mm^2 (0.4/2.8); $P = 0.005$]. Approximately 60% of BrdU-labeled cells differentiated into neurons as indicated by MAP-2 labeling. Cells stained also positive for the neuronal markers TUC-4 and beta-tubulin.

Conclusion: In a mouse model of *S. pneumoniae* meningitis, proliferation of neural progenitor cells was enhanced in the subgranular layer of the dentate gyrus. Cells differentiated into mature neurons as indicated by expression of TUC-4, MAP-2 and beta-tubulin. Endogenous repair mechanisms may limit the consequences of neuronal destruction after meningitis.

O155 Alveolar macrophage depletion in murine pneumococcal pneumonia

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Objectives: Alveolar macrophages (AM) contribute to the host defence against pulmonary pathogens. The role of AM in host defence was examined in a murine model of resolving pneumococcal pneumonia.

Methods: Pneumonia was induced in adult female C57BL6 mice by intratracheal instillation of 104 colony forming units (cfu) of type 1 *Streptococcus pneumoniae* (Spn). PBS was instilled as control. Depletion of AM was with intranasal pulmonary delivery of liposome encapsulated clodronate 48 h prior to Spn infection (AM–). Controls (AM+) were similarly treated with PBS (PBS-AM+) or PBS-filled liposomes (lip-AM+). 24 h after Spn infection bronchial alveolar lavage fluid (BALF) was collected. Numbers of cells in BALF were counted by hemocytometer, and cell type determined by light microscopy of cytospins. Bacterial load was determined in lung homogenates and blood. Apoptosis was detected by Annexin-V staining and morphology.

Results: AM depletion was 64% at 72 h, $P < 0.05$ vs. PBS-AM+ and enhanced AM apoptosis was observed in AM–. Spn recovered from the lung was greater in AM– (median (25%,75% percentile), 6×103 (1.5×103, 5×104)) than AM+ (PBS-AM+ 0 (0, 83); lip-AM+ 2.5×102 (0, 1.3×103)), $P < 0.05$ vs. PBS-AM+. Bacteremia was observed in 1/7 AM–, 0/8 PBS-AM+ and 1/8 lip-AM–. There was a greater number of polymorphonuclear leukocytes (PMN $\times$ 103) in Spn treated AM– mice 12.8 (6.7, 33.4) than AM+ groups; PBS-AM+ 1.8 (0.8, 2.5), $P < 0.05$ vs. AM+, or lip-AM+ 3.1 (1.1, 14.5)), which were similar to non infected groups (AM– 1.5 (0.6, 4.1); PBS-AM+ 2.7 (1.9, 3.6); lip-AM+ 3.3 (3, 5.7)).
Conclusion: AM depletion was associated with reduced bacterial clearance at 24 h, and increased PMN influx suggesting AM contribute to host defence in the lung against Spn.

O156 Treatment of experimental sepsis by multidrug-resistant *Pseudomonas aeruginosa* with thalidomide

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Objectives: The increased prevalence of nosocomial infections by multidrug resistant (MDR) isolates renders the necessity for effective immunomodulation. Thalidomide, that reduces half-life of mTNFa (Calabrese et al. Am J Med 2000, 108: 487), was applied in an experimental model of sepsis by MDR *P. aeruginosa*.
Methods: Sepsis was induced after the intraperitoneal injection of an 8log10 inoculum of one MDR isolate of *P. aeruginosa* in 64 male Wistar rats. The isolate was resistant to ceftazidime, imipenem, ciprofloxacin and amikacin. Thalidomide powder diluted in seed oil was administered by an orogastric catheter at a dose of 50 mg/kg in 24 rats 30 min before bacterial challenge. At the same time interval seed oil was administered by the same route in 20 rats; 20 rats served as controls. Survival was recorded and cumulative survival was estimated by Kaplan–Meier analysis. Comparisons were performed by log-rank test.
Results: Mean $\pm$ SE of survival of controls was 18.60 ± 1.84 h and of animals administered seed oil 12.60 ± 0.60 h (p: 0.036 compared to controls). No animal-control remained alive after 36 h and no animal administered seed oil remained alive after 24 h. Mean $\pm$ SE of survival of animals treated with thalidomide was 30.50 ± 6.62 h ($P < 0.001$ compared to both other groups). No animal treated with thalidomide remained alive after 144 h.
Conclusions: Pre-treatment with thalidomide delayed considerably the advent of sepsis and of subsequent death in an experimental model of sepsis by MDR *P. aeruginosa*. These results merit further clinical evaluation.

O157 Early alterations of serum oxidant potential in the pathogenesis of sepsis by multidrug-resistant *Pseudomonas aeruginosa*

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Objectives: To investigate the correlation of the early changes of the redox potential to the alteration of antioxidant status, on experimental sepsis by a multidrug- resistant *P. aeruginosa*.
Methods: Seven male rabbits were applied. Sepsis was induced after the intravenous administration of one multidrug-resistant *P. aeruginosa* isolate from a catheter inserted into the right jugular vein. Blood was sampled at regular time intervals for the determination of the concentrations of malondialdehyde (MDA), of total antioxidant status (TAS) and of tumor necrosis factor (TNFa). The concentration of MDA was estimated by the thiobarbiturate assay and of TNFa by the cytotoxic influence of serum on the cell line L929 from fibroblasts. The determination of TAS was assessed by a chromometric method depending from the time and temperature.
Results: Mean $\pm$ SD of MDA was 10.80 ± 4.29 M before bacterial inoculation; it was changed to 12.70 ± 7.92, 14.7 ± 3.08, 15.73 ± 2.68, 13.8 ± 2.94, 17.22 ± 0.22 and 11.42 ± 4.45 M at 15, 30, 60, 120, 150 and 210 min after bacterial inoculation, respectively. Mean $\pm$ SD of TNFa was 93.3 ± 52.2, 76.1 ± 40.9, 59.0 ± 46.2, 210.0 ± 105.2 and 210.0 ± 105.2 pg/mL 30, 60, 120, 150 and 210 min after bacterial inoculation, respectively. Mean $\pm$ SD of TAS was 1.54 ± 0.19 mM before bacterial inoculation; it was changed to 1.38 ± 0.54, 1.16 ± 0.90, 1.26 ± 0.28, 1.45 ± 0.57, 1.36 ± 0.85 and 1.59 ± 0.11 mM at 15, 30, 60, 120, 150 and 210 min after bacterial inoculation, respectively. A positive correlation was found between concentrations of MDA and TAS ($P = 0.017$).
Conclusions: Analysis of results indicates the significant and early augmentation of the redox potential during experimental sepsis by multidrug- resistant *P. aeruginosa*. That increase seems to correlate with equivalent alterations of antioxidant status, leading to the conclusion that the intrinsic antioxidant mechanisms of resistance are activated in an effort to restrain the septic process. Future studies on the administration of antioxidant therapy on the early course of sepsis are mandatory.

O158 *Streptococcus pyogenes* virulence genes might predict outcome

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Objective: Group A streptococcal (GAS) meningitis is a rare but serious condition, representing about 2% of all invasive GAS infections. A number of protein-binding factors (PBFs), facilitating bacterial adherence to certain host tissues, have been identified. In this study we assessed the genetic make-up of these PBFs and exotoxins in GAS isolates from meningitis patients.
Methods: All GAS meningitis patients between 1992 and 1995 in the Netherlands were registered. The occurrence of death or systemic extra-neurological complications was evaluated. All patient-isolates were subjected to T-serotyping and M-genotyping, and were tested for nine exotoxin genes and 11 PBF-genes by PCR. We assessed the correlation between T/M type, toxin/PBF-gene profile and clinical outcome.
Results: Twenty-seven patients with clinically and microbiologically defined GAS meningitis were included. Ten out of 26 patients, evaluated for clinical outcome, developed extraneurological complications, including two patients who died. The predominant M-types were M1 (9/27), M6 (5/27) and M3 (3/27). The nine genes encoding exotoxins *speA/speB/speC/speF/speG/speH/speJ/ssa* and *smeZ* were found in 48; 100; 33; 100; 93; 4; 37; 30 and 37% of all isolates, respectively. The 11 PBF genes, i.e. *cpa/cpa-1/fbp-54/prtf-1/prtf-2/prtf-15/sfb/fba/pfbp/sciA/sciB* were detected in 4; 26; 30; 19; 4; 4; 11; 33; 4; 59 and 89% of all isolates, respectively. *Fba*, *fbp-54*, *cpa-1* and *speJ* were only identified in M1 isolates. *SpeA* was only recognized in either M1 or M3 types and *prtf-15* was exclusively associated with M6.
Conclusion: Ssa, which was not related to any M-type, was the only factor correlating to the occurrence of complications or death (RR = 4; $\chi^2 = 4.40$ ($P < 0.05$)). The apparent clinical significance of virulence genes in *streptococcus pyogenes* warrants further research.

The case for getting it right first time in nosocomial infections (Symposium arranged by AstraZeneca)

S159 Introduction from the chairman: the case for 'getting it right first time' in nosocomial infections

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The incidence of nosocomial infections has increased steadily over 25 years. These infections are a serious threat to hospitalized patients and are associated with increased rates of mortality, morbidity, duration of hospitalization and intensive care unit stay, and considerable cost impact. Most nosocomial infections arise from invasive procedures, presence of indwelling devices and cross-transmission on the hands of medical personnel. Bacteria frequently implicated include enteric bacilli (*Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp.), *Staphylococcus aureus* (particularly methicillin resistant strains), coagulase-negative *Staphylococcus*, nonfermentative Gram-negative bacilli (*Pseudomonas aeruginosa* and *Acinetobacter* spp.) and enterococci. Recently, the level of

antimicrobial resistance has increased sharply among these pathogens, reducing the effectiveness of many classes of antimicrobials used to treat these infections. There is considerable evidence to support the concept that 'getting it right first time' – by using appropriate empiric antimicrobial therapy early in the course of a nosocomial infection – reduces mortality, morbidity, and overall costs. Selection of appropriate antimicrobial therapy needs an accurate diagnosis and assessment of patient risk factors. We need a thorough understanding of the microbial cause, anticipated resistance patterns of the infecting organisms and the properties of the antimicrobials available for treatment, namely, spectrum of activity and potency (including against resistant strains), pharmacokinetic and tolerability/safety data. Appropriate initial treatment may be the broadest spectrum monotherapy or a combination regimen which should be amended when results from the cultures and susceptibility tests become available. This strategy can improve clinical outcome, reduce costs and may help to reduce the emergence of resistance within the microbiological environment. This symposium will examine the importance of appropriate initial antimicrobial therapy in the management of nosocomial infections, the reasons for inappropriate initial therapy, and the consequences. Using case histories, a panel of multidisciplinary experts will examine the current and optimal management of two common nosocomial infections – nosocomial pneumonia and surgical infections – and discuss strategies to try to increase the frequency with which we 'get it right first time'.

S160 Making the first choice the right choice

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Nosocomial infections are associated with significant morbidity and mortality. It is critical that they are treated appropriately by initiating suitable antimicrobial treatment early in the course of infection. Usually, this means starting with the broadest spectrum monotherapy until the infecting pathogen is identified, then reducing the antimicrobial spectrum. There may be circumstances when a narrower spectrum antimicrobial is appropriate initial therapy. This will allow successful treatment of high-risk patients and avoid unnecessary antibiotic use and resistance. In a recent study of patients with infections requiring admission to the medical/surgical ICU, we found that patients receiving adequate antimicrobial therapy had a significantly ($P < 0.001$) lower rate of hospital mortality (12% vs. 52%; RR = 4.26) and infection-related mortality (18% vs. 42%; RR = 2.37) than those receiving inadequate antimicrobial treatment; inappropriate antimicrobial treatment was the most important independent determinant of hospital mortality. In this study, inadequate antimicrobial treatment was defined as ineffective treatment of a microbiologically documented infection at time of identification, either through absence of antimicrobials directed at one of the underlying pathogens, or use of an agent to which the causative pathogen was resistant. Among patients with nosocomial infections in this study, lack of coverage for Gram-negative bacteria resistant to the administered third-generation cephalosporins accounted for most instances of inadequate antimicrobial treatment. Risk factors for receiving inadequate therapy included prior exposure to antibiotics, prolonged length of hospital stay, presence of invasive devices, presence of a bloodstream infection, increasing APACHE II score and decreasing patient age. Other investigators have also demonstrated a statistically significant association between the initial administration of adequate empiric antimicrobial treatment and reduced hospital mortality in patients with ventilator-associated pneumonia (VAP) and patients with nosocomial blood stream infections (Table 1). Inappropriate antimicrobial therapy has also been shown to increase mortality in patients with other serious infections such as postoperative peritonitis. As well as reducing mortality, early use of adequate empiric antimicrobial therapy has been shown to significantly reduce duration of hospital stay and time in the ICU, which is an important resource consideration.

Table 1

		Mortality		
Study	Patient type (no.)	Adequate antimicrobial therapy %	Inadequate antimicrobial therapy %	*P*
1	Medical/surgical ICU ($n = 2000$)	12.2	52.1	<0.001
2	Mechanically ventilated nosocomial pneumonia ($n = 50$)	37.5	91.2	<0.001
3	VAP ($n = 430$)	16.2	24.7	<0.04
4	VAP ($n = 113$)	15.4	37.0	<0.05
5	Bloodstream infections ($n = 492$)	28.4	61.9	<0.001
6	Bloodstream infections ($n = 3413$)	20.0	34.0	<0.0001

Performance by design – developing solutions for efficacy against drug-resistant *Streptococcus pneumoniae* (Symposium arranged by GlaxoSmithKline)

S163 Standards of antibacterial performance

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Drug development and clinical use of antibiotics continues to evolve. While antibiotic resistance remains an important determinant for drug development and therapeutic choice, pharmacokinetic and pharmacodynamic parameters are having an ever-increasing influence on dosage regimens. Recently, licensed new therapies are largely directed at serious hospital-associated Gram-positive infections. However, in the community, therapeutic choice is largely dependent on well established agents from limited classes of antibiotics. In order to maximize benefits from such agents, it is appropriate that dosage regimens be reviewed in the light of new knowledge, particularly in the area of pharmacokinetics and pharmacodynamics. This symposium will address the scientific and clinical data that support the design and development of a new pharmacokinetically enhanced formulation of amoxicillin/clavulanate.

S164 Elements of design: the knowledge on which to build

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Pharmacodynamics is a tool to enable antibiotic dosing to be optimized in terms of pathogen clearance and prevention of resistance. This applies to susceptible wild-type bacterial populations and those strains which have known mechanisms of resistance. For most antibiotic drug classes, a combination of animal and in vitro evaluations have resulted in definition of the dominant pharmacodynamic index for efficacy, the AUC/MIC, C_{max}/MIC or T > MIC. For β-lactams, the T > MIC is the index that is best correlated to bacterial efficacy. The magnitude of the pharmacodynamic index required for bacteriological cure depends on the species treated, family of β-lactam studied, drug protein binding, immune status of the host and degree of bacterial clearance required. Larger T > MIC values are required to kill bacteria that have a static effect. Defining the magnitude of the pharmacodynamic index also helps to define the values for clinical breakpoints and hence the categorization of strains as clinically sensitive or resistant. The T > MIC for any agent can be increased by larger doses, more frequent dosing or provision of slow release formulations. Such approaches may mean that bacteria previously 'resistant' will now respond to therapy. Clinically, important resistance is therefore not an absolute phenomenon but is greatly influenced by drug formulation and dosing regimen.

S165 Building in efficacy: developing solutions to combat drug-resistant *Streptococcus pneumoniae*

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The development of our understanding of the pharmacokinetic (PK) and pharmacodynamic (PD) principles that determine antimicrobial efficacy has advanced substantially over the last 10 years. We are now in a position to use PK/PD principles to set targets for antimicrobial design and optimization so that we can predict eradication of specific pathogens or of resistant variants when agents are used clinically. Optimization of PK/PD parameters to

enable the treatment of resistant pathogens may not be possible with many current agents. For example, this is difficult to achieve with many oral cephalosporins due to lack of dose linearity of PK or the need for substantial changes to the PK profile to achieve adequate time above MIC (T > MIC) for penicillin-nonsusceptible pneumococci. For macrolides, PK/PD parameters are adequate against macrolide-susceptible pneumococci, but not against macrolide-resistant strains with efflux or ribosomal-methylase mediated resistance, or against *Haemophilus influenzae*, which has intrinsic macrolide efflux mechanisms. While older quinolones, such as ciprofloxacin had inadequate PK/PD profiles against pneumococci, newer quinolones meet the required PK/PD targets against quinolone-susceptible pneumococci, but not against all quinolone-resistant strains. Aminopenicillins, such as amoxicillin, have linear PK and have a good safety profile even at high doses. Although current formulations of amoxicillin and amoxicillin/clavulanate have retained efficacy against most, but not all, penicillin-nonsusceptible *Streptococcus pneumoniae*, additional coverage is required to combat the growing problem of drug-resistant strains. The new pharmacokinetically enhanced oral formulation of amoxicillin/clavulanate, 2000/125 mg b.i.d. (AUGMENTIN XR^{TM}), was designed using PK/PD principles to be able to eradicate *S. pneumoniae* with amoxicillin MICs of up to at least 4 mg/L, which includes most penicillin-resistant isolates. For amoxicillin and amoxicillin/clavulanate, a T > MIC of 35–40% of the dosing interval (based on blood levels) is predictive of high bacteriological efficacy. This target was met by the design of a unique bilayer tablet incorporating 437.5 mg of sustained-release sodium amoxicillin in one layer plus 562.5 mg of immediate-release amoxicillin trihydrate and 62.5 mg of clavulanate potassium in the second layer, with two tablets administered for each dose. This unique design extends the bacterial killing time by increasing the T > MIC to 49% of the dosing interval against pathogens with MICs of 4 mg/L, and 35% of the dosing interval against pathogens with MICs of 8 mg/L. Based on these results, this new amoxicillin/clavulanate formulation should be highly effective in treating respiratory tract infections due to drug-resistant *S. pneumoniae* as well as β-lactamase-producing pathogens, such as *H. influenzae* and *Moraxella catarrhalis*.

S166 Proof of concept: performance testing in models

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Since time above MIC (T > MIC) is the pharmacokinetic/pharmacodynamic (PK/PD) parameter correlating with efficacy of amoxicillin against *Streptococcus pneumoniae*, studies were performed in the neutropenic mouse-thigh infection model to determine the percentage of time the serum concentrations need to exceed the MIC for susceptible strains and resistant ones with MICs of up to 8 mg/L. The duration of time required for a bactericidal effect (at least a 2-log kill) was similar for all strains and varied from 35 to 40% of the dosing interval. The new pharmacokinetically enhanced formulation of amoxicillin/clavulanate (AUGMENTIN XR^{TM} 2000/125 mg b.i.d.) has a T > MIC of 49% against bacterial strains with MICs of 4 mg/L, and 35% against strains with MICs of 8 mg/L, which exceed the >35–40% predictive of high bacteriological efficacy. In vitro models provide a difficult test for antimicrobial efficacy due to the absence of a host immune response, so efficacy in immunocompetent humans would be expected to be higher. However, in in vitro models, amoxicillin/clavulanate 2000/125 mg b.i.d. achieves significant bacterial killing against strains of *S. pneumoniae* with MICs of 4 mg/L and in some strains with MICs of 8 mg/L. Animal models, such as the rat model of respiratory tract infection, do take into account host immune effects and are therefore a more accurate indicator of bacteriological efficacy in humans. In addition, dosing in the rat is adjusted to simulate the pharmacokinetics of dosing in humans. In a rat model of pneumonia, amoxicillin/clavulanate 2000/125 mg was highly effective against *S. pneumoniae* strains with amoxicillin MICs of 4 or 8 mg/L. Against strains with amoxicillin MICs of 4 mg/L, amoxicillin/clavulanate 2000/125 mg b.i.d. was significantly more effective than the conventional 875/125 mg b.i.d. formulation, azithromycin and levofloxacin, even though all levofloxacin MICs were $\leq$1 mg/L. Following infection with *S. pneumoniae* strains with amoxicillin MICs of 8 mg/L, the amoxicillin/clavulanate 2000/125 mg b.i.d. formulation was more effective than the conventional amoxicillin/clavulanate formulations 875/125 t.i.d. and b.i.d. and 1000/125 mg t.i.d., and had similar or better efficacy than azithromycin or levofloxacin, depending on the strain. These data indicate the potential benefit of therapy with amoxicillin/clavulanate 2000/125 mg b.i.d. compared with conventional formulations and other marketed antimicrobials in the treatment of respiratory tract infection and, if confirmed by clinical trials, supports a susceptible breakpoint of at least 4 mg/L.

S167 Performance in practice: bacteriological efficacy in patients with drug-resistant *Streptococcus pneumoniae*

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Using pharmacokinetic/pharmacodynamic (PK/PD) principles, amoxicillin/clavulanate (AMX/CA) 2000/125 mg b.i.d. (AUGMENTIN XR^{TM}) was designed to provide adequate levels of AMX over the 12-h dosing interval to eradicate penicillin-resistant *Streptococcus pneumoniae* (PRSP, penicillin MICs $\geq$ 2 mg/L) with AMX MICs of at least 4 mg/L. The efficacy of AMX/CA 2000/125 mg in patients with RTIs caused by *S. pneumoniae*, including isolates with elevated PEN (2–16 mg/L) MICs, was evaluated. Data from 10 clinical studies were combined: seven randomized (1 : 1), double-blind, controlled trials (ITT, $n = 3377$) [AMX/CA 2000/125 mg b.i.d. vs. levofloxacin 500 mg o.d. in acute bacterial sinusitis (ABS); levofloxacin 500 mg o.d. in acute exacerbations of chronic bronchitis (AECB); clarithromycin 500 mg b.i.d. in AECB; AMX/CA 875/125 mg b.id./t.i.d. and 1000/125 mg t.i.d. in community-acquired pneumonia (CAP)] and three noncomparative studies (ITT, $n = 3024$) (two in ABS, one in CAP). The bacteriological per-protocol (PP) population at follow-up (FU, Day 14–39) comprised 1295 patients for AMX/CA 2000/125 mg and 241 for comparators. With AMX/CA 2000/125 mg at FU, outcome was successful (clinical success and eradication/presumed eradication) in 85/90 (94.4%) patients with *S. pneumoniae* in comparative studies and 419/443 (94.6%) in noncomparative studies (94.6% success overall), and with comparators 59/70 (84.3%) were successes. In the AMX/CA 2000/125 mg group at FU, 53/535 *S. pneumoniae* isolates were resistant to PEN. At FU, 51/52 (98.1%) patients with PRSP were successes, including 7/7 with AMX MICs of 4 mg/L and 7/8 with AMX MICs of 8 mg/L. There were six PRSP isolates in the comparator group (two isolates were from one patient), and 3/5 patients in this group were successes. In conclusion, AMX/CA 2000/125 mg demonstrated clinical success against 51/52 patients with PRSP, including 14/15 strains with AMX MICs of 4–8 mg/L. These results for the PK-enhanced formulation of amoxicillin/clavulanate 2000/125 mg are in line with the high efficacy against PRSP predicted using PK/PD parameters.

Emerging techniques for the rapid diagnosis of infectious diseases

K175 Emerging techniques for the rapid diagnosis of infectious diseases

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High-throughput systems for analysis of genetic variability and affinity-reagent based proteomics have been set-up based on 'in-house' designed robotic workstations. The analysis of single nucleotide polymorphisms is based on pyrosequencing (Ronaghi, Uhlen and Nyren, Science 1998; 281: 363–5). Microfluidic applications will be discussed suitable for bacterial and viral typing as well as analysis of antibiotic or antiviral resistance. New approaches for in vitro selection of affinity reagents will also be discussed. The affinity reagents are based on affibodies (Nord et al. Nature Biotechnology 1997; 15: 772–7) allowing robust 'artificial antibodies' suitable for applications such as protein 'chips'.

Carbapenem-hydrolyzing beta-lactamases: a last frontier for beta-lactamases?

S176 Zn-carbapenemases: the ultimate danger?

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Carbapenems exhibit very distinct behaviors in the presence of the various classes of beta-lactamases. Imipenem acylates most class A enzymes slowly and deacylation is also slow, so that it behaves as a sluggish substrate. Class C enzymes are more rapidly acylated but again, deacylation is slow. In these cases, the enzyme is transiently immobilized as an acylenzyme. Some class D enzymes appear to hydrolyze carbapenems, but this activity is still poorly characterized. The metallo-beta-lactamases (MBLs, class B) usually behave as efficient carbapenemases. Of particular interest are the class B2 enzymes produced by *Aeromonas* whose activity seems to be restricted to carbapenems. When compared to those of other MBLs, the sequences of these enzymes exhibit some specific residues including one of the Zinc ligands, an Asn replacing the His116 found in enzymes of classes B1 and B3. The N116H mutation, which restores the situation found in the latter enzymes decreases the carbapenemase activity but does not restore the broad specificity spectrum characteristic of class B1. The additional N220G mutation further decreases the carbapenemase activity but strongly increases that against all other sustrates, which yields a broad spectrum enzyme with a rather low but significant activity. Residue 220 is situated just before the Cys 221 which is an important Zn ligand in subclasses B1 and B2. A characteristic of the class B enzymes of known structure is the presence of a 'flexible flap' near the active site. The influence of this flap in subclass B1 has been studied by site-directed mutagenesis. It mainly affects the binding of several substrates, but relatively less so for carbapenems. Up to the present time, several metallo-beta-lactamase have been found to significantly hydrolyze most clinically useful beta-lactams with the sole exception of aztreonam. To fight these enzymes, it is thus quite important to try to discover specific inhibitors which are not general Zn-ligands.

S177 Phylogenesis and epidemiology of the metallo-beta-lactamases: a guided tour

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Objectives: Metallo-beta-lactamases (MBL) were discovered later than serine-beta-lactamases and are mechanistically and evolutionarily unrelated to the latter enzymes. Notable features of MBL are the potent carbapenemase activity, a usually broad substrate profile, and resistance to conventional inhibitors. Although MBL are less prevalent than serine-beta-lactamases in the clinical setting, the number of known MBL has increased steadily during recent years, and these enzymes are currently regarded as emerging resistance determinants of increasing relevance in gram-negative pathogens. The objective of this presentation is to provide an updated overview on the epidemiology and the origin of these enzymes.
Methods: Results of experimental data from our laboratory and data from the scientific literature will be integrated to elaborate an overview on issues concerning evolution and epidemiology of MBL.
Results: the MBL family (class B) currently includes at least 19 different enzymes, with multiple allelic variants known for most of them. Members of this family can be divergent from each other by more than 90% of the amino acid sequence, but retain a conserved fold. Structural diversity is associated to considerable functional diversity within the family. According to structural similarities MBL are clustered in three different subclasses (B1, B2, and B3), and appear to be evolutionarily related to other proteins of unknown or diverse diverse functions (e.g. glyoxalase, esterase, sulfatase). MBL have been detected in species of phylum XII (Proteobacteria), XIII (Firmicutes) and XX (Bacteroidetes), and are encoded either by resident chromosomal genes or by genes acquired by recent horizontal transfer. From the clinical standpoint, the most relevant are the IMP- and VIM-type enzymes, that exhibit an exceedingly broad susbtrate specificity and are encoded by mobile elements (integron-borne gene cassettes). These enzymes are spreading among Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter* spp. and other gram-negative nonfermenters and, to-date, have been reported in at least three continents (Asia, Europe and the Americas).
Conclusions: MBL constitute a growing family of enzymes of increasing importance. Secondary MBL are now widespread and could become a global problem. Efficient monitoring of these enzymes is essential to understand their epidemiology and to develop strategies for containment. Work supported in part by EU contract HPRN-CT-2002–00264.

S178 Carbapenemase activity of nonmetallo enzymes

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Whereas most of the carbapenem-hydrolyzing beta-lactamases belong to the group of Ambler class B metallo-enzymes, several beta-lactamases belonging to the Ambler class A and class D have extended spectrum to carbapenems. A few clavulanic acid-inhibited class A beta-lactamases possess a substrate profile extended to carbapenems and aztreonam. This is the case of the chromosomally encoded NMC-A, IMI-1, and SME-1/−2 enzymes that are from rare clinical isolates of *Enterobacter cloacae* and *Serratia marcescens* in Europe and the USA and more recently from *Enterobacter sakazakii* isolates from US rivers. The clavulanic-acid inhibited beta-lactamase KPC-1 represents a more important threat since it is plasmid-mediated and has a spectrum of activity that include carbapenems and expanded-spectrum cephalosporins. It has been reported from *K. pneumoniae* from three cities in the USA with one major outbreak. A variant of the plasmid- and integron-located extended-spectrum beta-lactamase GES-1 gene, blaGES-2 was recently found in *P. aeruginosa* in South Africa. It has hydrolysis spectrum extended weakly to carbapenems and GES-2-producing strains have been involved in a nosocomial outbreak. The chromosome-encoded SHV-38 enzyme in *K. pneumoniae* is a point-mutant derivative of the narrow-spectrum SHV-1 enzyme and is the only example of a SHV-type enzyme with low level extension of its spectrum to imipenem. Some of the growing carbapenem resistance in *Acinetobacter* spp. is associated with weak Ambler class D carbapenemases that are mostly chromosome-encoded. These enzymes belongs to two clusters, one of them (OXA-24-related) associated with Iberia and the other (OXA-23-related) more scattered. A novel plasmid-encoded enzyme, OXA-42 has been discovered recently from a Turkish *K. pneumoniae* isolate. It has strong activity against carbapenems.

S179 Slow hydrolysis and permeability deffects

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Seville, E

Expression of β-lactamases that hydrolyze carbapenems with low efficiency may lead to carbapenem resistance in strains showing decreased permeability, usually because of porins loss. Modified lipopolysaccharide has also been implicated in resistance to carbapenems, but its actual contribution need additional studies. In strains producing efficient carbapenemases, altered permeability increases the level of resistance. Hyperproduction of chromosomal AmpC (which hydrolyzes carbapenems poorly) plus porin loss has been related to carbapenem resistance in enterobacteria, particularly *Enterobacter* spp., *Citrobacter freundii* and *Serratia marcescens*. Blocking AmpC activity abolishes resistance to carbapenems, and revertants re-expressing lost porins also become carbapenem-susceptible. Imipenem permeates faster than meropenem through outer membrane, but the highest stability of meropenem to most β-lactamases usually translates into lower MICs of the latter. In some enterobacteria (Proteus, . . .) resistance to carbapenem has been described in strains deficient in an outer membrane protein, but the lost protein seems not to be a porin, and the resistance is more likely associated to PBP changes. *Klebsiella pneumoniae* strains producing extended-spectrum beta-lactamases (EBSL) are susceptible to carbapenems, even when the two major porins (OmpK35 and OmpK36) of the species are lost, because these agents are (with a few exceptions) poor substrates of ESBL. *K. pneumoniae* may become resistant to carbapenems because of either carbapenemase production or the combination of porin loss plus plasmid-mediated AmpC (p-AmpC) production. Carbapenem-resistance in *Escherichia coli* has also been related to porin loss and production of p-AmpC. The role of minor enterobacterial porins (OmpK37 of *K. pneumoniae*, . . .) in carbapenem resistance is still poorly known. In *Pseudomonas aeruginosa* loss of porin OprD in strains producing

AmpC causes resistance to imipenem, and decreases the activity of meropenem. A similar finding has been observed in strains producing specific carbapenemases such as VIM-1. Carbapenems that penetrate using OprD-independent pathways show good in vitro activity against AmpC(+)/OprD(–) *P. aeruginosa*, supporting the importance of the coordinated expression of both mechanisms. In *Acinetobacter baumannii* the importance of altered permeability as a mechanism involved in carbapenem-resistance has still to be proved. New data on the regulation of bacterial permeability (for example the recently described role of Zn in regulation of OprD, or the relationship between porin loss and active efflux in *K. pneumoniae*) will contribute to our understanding of the role of permeability on the resistance of Gram-negative bacteria to carbapenems.

Harmonization of quinolone breakpoints in Europe (Symposium arranged with EUCAST)

S181 Breakpoints – the EMEA experience

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The European Medicines Evaluation Agency and its scientific committee for proprietary medicinal products (CPMP) evaluates new medicinal products in the centralized procedure. After a recommendation by the CPMP a marketing authorization that covers the whole EU is obtained. Since the EMEA operations started in January 1995 only three antibacterial products (ertapenem, telithromycin and trovafloxacin) have been licensed via the EMEA. Many applicants have preferred to go the national routes for marketing authorization applications including those for many antibiotics giving them the opportunity to focus on specific markets if they wish. This may partly be due to a lack of harmonization in the evaluation of the benefits and risks for antibacterial agents. The CPMP efficacy working partly in recognition of the need for harmonized assessments has developed three guidelines on the evaluation of antibacterial products, on the use of PK/PD data in drug development for antibacterials and on the information that should go into the harmacodynamic section of the SPC. In the latter case, an ongoing revision of the guidelines recognizes the existence of national and European bodies for setting breakpoints. Collaboration on the EU level in setting these breakpoints for new antibacterial products would facilitate EU harmonization and could benefit EU regulatory assessment of new antibacterial agents.

S185 Harmonization of fluoroquinolone breakpoints in Europe

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The European Committee on Antimicrobial Susceptibility Testing (EUCAST), convened by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) together with the national breakpoint committees in France, Germany, Norway, Sweden, The Netherlands and United Kingdom, has started a process for harmonizing European antimicrobial breakpoints. This process will be presented by EUCAST and commented on by the European Medicines Evaluation Agency (EMEA). The EUCAST has agreed on definitions for clinical breakpoints and epidemiological cut-off values for the measurement of antimicrobial resistance development. We have furthermore gathered a large number of MIC distributions from many sources which, apart from being used in the process of setting breakpoints and epidemiological cut-off values, will be available to all via the EUCAST internet website (http://www.eucast.org). The process by which EUCAST sets clinical and epidemiological breakpoints for new and older drugs will be presented. It involves evaluation of clinical data, microbiological data, PK/PD data and Monte Carlo simulation. EUCAST has defined harmonized tentative clinical breakpoints and decided on epidemiological cut-off values for the fluoroquinolones. These will be presented together with wild-type MIC distributions and a new software for accessing the MIC distributions.

Meningococcal disease

S186 Recent developments in pathogenesis and clinical practice

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There have been numerous advances in diagnosis and management of meningococcal disease over the past 3 years. Our knowledge of the pathogenesis of this disease has also increased considerably. The presentation, severity, and compartmentalization of the infection varies widely between patients. There are new algorithms to aid diagnosis in patients with undifferentiated disease, and the use of PCR has permitted nonculture diagnosis and will, in the future, permit rapid diagnosis and typing. There have been worrying developments in antimicrobial sensitivity to beta-lactams so that in some regions of Europe, efficacy of penicillin can no longer be universally assumed. Dealing with the potential community and nosocomial dissemination of the infection has also been made easier by improvements in our understanding of transmission dynamics. Disease occurs when the meningococcus translocates from the nasopharynx to the bloodstream and disseminates. The virulence factors which determine the success of the pathogen in each of these environments have been partly established but we still do not fully understand which host and microbial factors interact to permit invasion and disease. There has been progress in human genetic susceptibility studies, and a number of gene variations that influence innate immunity which are associated with susceptibility and severe meningococcal disease have been determined.

S188 Molecular epidemiology and typing of *Neisseria meningitidis*

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Meningococcal disease remains an important cause of morbidity and mortality world wide. Despite a long history of research, dating back to the earlier part of the last century, comprehensive vaccines against *Neisseria meningitidis*, the etiologic agent of meningococcal disease, are yet to be developed. Attempts to control meningococcal disease are confounded by meningococcal diversity and epidemiology, with disease outbreaks occurring sporadically and unpredictably notwithstanding the frequent occurrence of this bacterium as a commensal in the upper respiratory tract of adult humans. Epidemiological studies, especially those that exploit molecular characterization techniques combined with evolutionary and population genetic approaches, have been highly informative in elucidating the biology of this paradoxical pathogen. Meningococcal populations sampled from the throats of healthy individuals are both genetically and antigenically highly diverse, while those recovered from disease are more limited in their diversity. It is know known that the great majority of disease in the latter half of the twentieth century was caused by as few as a dozen major disease-associated genotypes, with six of these being predominant. Different genotypes tend to be associated with different epidemiological characteristics and with certain antigenic components, especially the capsular polysaccharide. Detailed genetic studies of meningococci from carriage and disease are beginning to reveal the reasons for

meningococcal diversity and to define ever more precisely the genotypes responsible for disease. These studies are also enabling the effects of population-scale immunization on the evolution of the meningococcus to be evaluated.

S189 Meningococcal surface components: implications for pathogenesis and vaccine development

T. Tønjum
Oslo, N

Neisseria meningitidis is the major cause of bacterial meningitis and septicemia worldwide. Its most important virulence factors are associated with the outer membrane, among them the capsule, pili, opa-proteins, porins and LPS. The expression patterns of genes encoding these components vary concomitantly, creating excessive amounts of meningococcal variants. Neisserial cell surface variation serves as an adaptive mechanism which can modulate tissue tropism, immune evasion and survival in the changing host environment. Genome alterations due to horizontal gene transfer and various recombinational events, as well as environmental DNA damaging agents, will constantly challenge the gene pool of *N. meningitidis*. Mechanisms for rapid genome variability, adaptability and maintenance are a necessity to ensure meningococcal fitness and survival. The unstable expression of surface components make meningococcal vaccine development an unusual challenge. Various surface exposed components should still be evaluated for meningococcal vaccine purposes. To be effective, a meningococcal vaccine must induce bactericidal and/or opsonic antibodies against one or more of the cell surface components expressed by *N. meningitidis*. The vaccine candidate must be sufficiently conserved and presented on the surface by all meningococcal strains. The capsular vaccines are relevant for serogroup A and C strains, however, the capsule of meningococcal serogroup B strains is poorly immunogenic. A number of other neisserial outer membrane components need to be considered for vaccine purposes to cover all serogroups; in addition to the virulence components listed, some of the current candidate structures are NspA, TbpA and various lipoproteins. The strategy to achieve novel and efficient vaccines must involve single component characterization as well as a combined genomic and proteomics-based approach; vaccine candidates must be tested for protective efficiency alone and in relevant combinations, such as in the naturally occurring outer membrane vesicles. The dynamics of meningococcal genomic changes and genome maintenance/DNA repair affect the net outcome in terms of DNA sequence variability and conservation. The effect of genome dynamics on meningococcal surface exposed virulence factors and vaccine candidates will be discussed.

Clostridium difficile disease: incidence and interventions (Symposium arranged with the ESGCD)

S190 Preventing cross-transmission of *Clostridium difficile*

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The incidence of *Clostridium difficile* (CD) among outpatients is relatively low. In the hospital setting, the acquisition rate is much higher and varies depending on the type of ward and the eventual presence of an epidemic situation. Contamination can occur from two separate sources. The first one is the emergence, following antibiotic treatment, of a strain already present in the gut in very low quantities, and the second one is the acquisition from contact with healthcare personnel workers hands, infected patients or their environment. Patients with CD-associated diarrhea (CDAD) typically have large numbers of organisms in their stools and constitute the main reservoir, although asymptomatic carriers also contribute to a lesser extent to the spread of CD. It has been shown that, in the case of CDAD, the patients' environment is rapidly contaminated by spores that can persist for several months despite extensive cleaning. The environment becomes a secondary reservoir. Hands of healthcare workers are frequently contaminated and are the main vector of transmission. Contaminated tools such as thermometers have also been incriminated. Beside all measures aiming at decreasing risk factors, interruption of horizontal transmission of CD is the main control measure. This is based on contact precautions and environmental disinfection. Clinical bacteriology laboratories and infection control units play a major role in implementing control measures as soon as a positive case is diagnosed. Contact precautions such as isolation or cohorting, handhygiene and gloving have been successful in limiting the transmission of CD in hospitals. There is no evidence that the use of gowns in itself is efficient because it is usually implemented along with other measures. There is considerable circumstantial evidence about the role of environmental disinfection in decreasing the spread of CD. Daily room environmental cleaning with a disinfectant has been demonstrated successful in reducing the numbers of new CDAD cases. Phosphate-buffered hypochlorite and solutions of aldhehydes have been shown to significantly reduce the ward contamination rate. A recent study provided evidence that, contrary to chlorine based products, cleaning agents may increase the sporulation capacity of CD. Although research on nosocomial CD has come a long way since the late 70 s, CD lives up to its name by continuing to be a difficult nosocomial infection to deal with in our hospitals.

S191 Reducing the burden of *Clostridium difficile*-associated diarrhea

T. Riley
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Clostridium difficile is the most common cause of diarrhea in hospital patients. The main risk factors for *Clostridium difficile*-associated diarrhea (CDAD) are exposure to antibiotics and exposure to the organism. Attention to infection control and antibiotic prescribing behavior are obvious approaches to prevention of CDAD at a hospital level. In our hospital a steady rise in incidence of CDAD was observed during the 1980s that was related to cephalosporin use. In 1998, a review of antibiotic prescribing by the hospital's Drugs and Therapeutics Committee resulted in an almost total ban of ceftriaxone. Ceftriaxone use (measured by annual cost) fell substantially from A$250 000 in 1998 to A$32 000 in 1999. This policy change was followed by a 50% drop in the number of CDAD cases in 1999, a decrease that persisted into 2000. These findings suggest that it is possible to control CDAD by regulating antibiotic prescribing in the hospital. For individual patients, many of whom have recurrent CDAD, probiotic therapy may have a place. Several papers have reported the use of Lactobacillus GG in children and adults with recurrent CDAD. We have carried out two studies with yoghurt containing lactobacilli. In the first of these, patients given clindamycin for furunculosis were randomized to receive either yoghurt or no yoghurt while on therapy. Although the sample size was small, those taking yoghurt had a significantly reduced incidence of diarrhea and CDAD. In the second study, over 650 hospital patients taking antibiotics were given yoghurt daily and the impact on CDAD assessed. Over a 3-month period there was a 50% reduction in both the incidence of symptomatic diarrhea and CDAD. *Saccharomyces boulardii* therapy is also suitable for patients with recurrent disease. Our experiences with *S. boulardii* are limited, however, so far, we have treated 25 elderly patients with recurrent CDAD with vancomycin plus *S. boulardii* (500 mg bd) for a week, followed by another 3 weeks of just *S. boulardii*. Twenty-three of the 25 patients responded with one patient suffering recurrence of disease and one patient lost to follow-up.

S192 Prevention of *Clostridium difficile* infection at the bedside

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Leeds, UK

Clostridium difficile infection is an iatrogenic disease and therefore in theory is at least partially preventable. Whilst most diagnosed cases are hospitalized patients, there may be a significant burden of unrecognized *C. difficile* infection in the community accounting for up to 28% of laboratory detected cases. While *C. difficile* diarrhea is more common in older patients (~80% of diagnosed cases affect in-patients aged ≥65 years), increasing age per se is not a risk factor for infection severity. Significant risk factors for severe *C. difficile* diarrhea include functional disability, cognitive impairment, and recent endoscopy. There are controlled prospective and/or retrospective data indicating that some broad spectrum antibiotics, including ureidopenicillins (e.g. piperacillin–tazobactam) and ciprofloxacin, are significantly less likely to induce *C. difficile* infection in comparison with cephalosporins such as cefotaxime. Effective control of *C. difficile* in the hospital requires both antibiotic control and prevention of environmental seeding and spread of the bacterium. Restriction of cephalosporin and/or clindamycin prescribing, and avoidance of excessive duration surgical antibiotic prophylaxis can be used to reduce nosocomial *C. difficile* infection. Feedback of data on rates of antibiotic prescribing and *C. difficile* infection should be the starting point to controlling hospital endemics. Infection control measures such as improved hand cleansing and use of tympanic or disposable thermometers have been demonstrated to be effective at reducing the incidence of nosocomial *C. difficile* infection. Surveillance data indicate that epidemic *C. difficile* strains exist, and that the major such strain can be widely distributed in the hospital environment, both as a cause and result of nosocomial diarrhea. Hence, efforts should be concentrated on the control of such epidemic strains, and source isolation of symptomatic patients is important to prevent their dissemination. There are new data on the effectiveness of environmental decontamination regimens, in particular hypochloirte-based cleaning to help control nosocomial *C. difficile* infection.

S193 Prevalence and epidemiology of *Clostridium difficile* in Eastern Europe

E. Nagy
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To day *C. difficile* is accepted as the causative agent of a large number of nosocomial diarrhea. Besides these, *C. difficile* probably plays a more important role than previously thought in community-acquired diarrhea after antibiotic treatment. The main pathogenic factors are A and B toxins, but more and more information are collected about the presence of variant strains lacking one or both toxins, or those with characteristic changes in their toxin genes and producing a binary toxin. The importance to isolate *C. difficile* from diarrhoeal patients, to characterize their toxins and to use comparable typing methods for epidemiological purposes, is well recognized in Western European countries, however, only few data are available from the Eastern part of Europe. Prevalence data for the *C. difficile* infections in hospital wards differ from country to country mainly due to the differences in the methodology and the interest of the clinicians to get a correct diagnosis of the nosocomial diarrhea. During a 1-year period in a university hospital in Szeged (Hungary) out of 570 diarrhea feces obtained from different wards 120 (21%) proved to be *C. difficile* toxin positive, using the cytotoxicity assay when the testing was initiated by the lab and out of 375 samples for which the physicians had requested examinations 58 (18.3%) was positive with a toxin A EIA detection method. During the next 6-month period when all watery feces samples obtained from the different wards were cultured for *C. difficile* and the toxin detection was carried out by the cytoxicity assay from the supernatant of the isolated strains, 29% of the samples were positive. Selected isolates were typed by PCR ribotyping, which revealed basic differences between the Hungarian isolates and those collected from different West European countries. Among the 15 different ribotypes found among the 65 isolates, 087 was the most predominant whereas 001 is the most frequently isolated ribotype among the isolates from other countries. Two nontoxigenic *C. difficile* strains showed a new type. Despite of the fact that toxin A negative/toxin B positive isolates appeared in many countries, in our laboratory out of 153 *C. difficile* strains none of them showed this toxin type. As PCR ribotyping is not used generally in different countries and many different typing methods are used, it is difficult to calculate to day the overall clonal spread of *C. difficile* in Europe.

War, famine and infectious diseases

S194 The bugs of war

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Birmingham, UK

Infections have been major influences on many military campaigns, not infrequently being the principal factor that has made the difference between victory and defeat. Examples include malaria and dysentery in South-East Asia in World War II, gas gangrene in the trenches in World War I, meningococcal infection in military recruits and sexually transmitted diseases in most campaigns. The advent of certain chemotherapeutic agents such as the sulfonamides, penicillin and antimalarial drugs has had profound influences on certain campaigns. The first part of this presentation will explore these factors and also other infections associated with war. The second part of the presentation will address the early history of bioterrorism.

S195 Medical preparations for defense against biological terrorism

E. Eitzen
Washington, DC, USA

The Department of Health and Human Services in the United States is engaged in an aggressive effort to develop and acquire the medical countermeasures necessary to protect and defend US citizens against biological terrorism. The speaker will discuss the history of biological terrorism, the ways that terrorists might use biological agents against us, and how these agents might affect the victims. He will briefly present some lessons learned from the antrax attacks which occurred in 2001 in the US, and discuss how those lessons might apply to future defensive efforts. Finally, he will offer insights into ways that countries might be better prepared to manage the consequences of such attacks and hopefully give potential perpetrators pause in their desire to use such potentially deadly agents against our societies.

S196 Arboviruses as potential bioterrorist weapons – implications for surveillance and control

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Tel Hashomer, IL

Recent large outbreaks of West Nile Virus (WNV) infection in Europe, North Africa, the Middle East and North America, have provided insight into the potential threat of arboviruses as bioterrorist weapons. Several features make these viruses attractive for bioterror. They are readily accessible. Outbreaks are difficult to identify in the early stages and once the virus has established itself in the environment, it can spread rapidly. The insect vectors can be extremely difficult to control. Furthermore, the disease usually affects both humans and animals, resulting in both suffering due to morbidity and mortality and substantial economic costs. In 2000, there was a large outbreak of WNV infection in Israel with 428 cases and 37 deaths. During the year following discharge from hospital, a further 39 patients died, yielding a mortality rate significantly higher than expected after controlling for age. In addition, many patients complained of persistent symptoms. These findings demonstrate the potential for significant long-term effects among those infected. The outbreak in the United States in 2002, which resulted in more than 4000 cases and 273 deaths, also caused significant morbidity and

mortality among a large variety of birds and animal species. In order to meet the challenge of early detection and monitoring of intentionally caused arbovirus outbreaks, greatly improved surveillance systems are necessary. This will require close collaboration between the public health, clinical and veterinary communities, as well as the authorities responsible for insect control. In addition, substantially increased investment is required to develop more effective vector control methods, new vaccines and therapeutic drugs.

Views on antibiotic use

O198 Antibiotic resistance prevention and control (ARPAC): results of a first questionnaire on hospital demographics, available data and policies

F. M. MacKenzie, J. Bruce, M. J. Struelens, H. Goossens, K. J. Towner, I. M. Gould on behalf of the ARPAC Steering Group, ARPAC Participating Hospitals

Objectives: ARPAC is a concerted action project funded by the Research Directorate-General of the EC. It aims to lay the foundations for a better understanding of the emergence and epidemiology of antibiotic resistance and to evaluate and harmonize strategies for prevention and control of antibiotic resistant pathogens in European hospitals. The first phase of the project set out to establish the demographic characteristics of the participating hospitals and their ability to provide necessary data.
Methods: In order to recruit hospitals to the project, 2476 ESCMID members were sent a 59-question, questionnaire. It asked for their willingness and ability to supply data on hospital demographics, susceptibility data for nine alert organisms (including MRSA, VRE and several resistant Gram-negatives), antibiotic consumption data and information on antibiotic and infection control policies.
Results: At the end of 2002, 266 hospitals had expressed an interest in participating in ARPAC. Data are presented only for the 235 European hospitals. Fourteen out of 16 EU countries and 20 out of 22 other European countries were represented. Hospital characteristics: 71% were teaching hospitals with an average of 805 total beds and 38 ICU beds. Susceptibility data and alert organisms: 82% hospitals had data on a computer database and 79% could exclude duplicate isolates, 74% had experienced an outbreak of at least one alert organism in the last 5 years and 62% carried out active screening for MRSA. Antibiotic consumption and demographic data: 74% hospitals stored their data on a computer database, 58% used the WHO ATC classification for antibiotics, 50% used defined daily doses, and 91% could supply the number of occupied bed days per year. Antibiotic policies: 33% hospitals reported the existence of a national antibiotic policy and 63% the existence of a local policy. Infection control policies: 33% hospitals reported the existence of a national infection control policy and 85% the existence of a local policy, 51% reported the existence of national guidelines for the isolation of patients with resistant bacteria and 78% the existence of local guidelines. Sixty-three percent typed ALERT organisms locally and 58% also used reference laboratories.
Conclusions: By collecting the above data, ARPAC will provide a broad picture of the epidemiology of antibiotic resistant nosocomial pathogens in European hospitals and the range of activities conducted for their prevention and control.

O199 Consumption of antibiotics in hospital care in Europe: first results of the ESAC retrospective data collection

M. Elseviers, M. Ferech, R. Vander Stichele, H. Goossens
Antwerp, B

Objectives: ESAC (European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2001), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: HC use data were provided sent by 22 countries; two were unsuitable for international comparison, due to late data delivery in an idiosyncratic format (CZ, PT). In six countries (BG, ES, HR, IT, LT, PL), sample (not census) data were collected.
Results: In 2001, HC use in Europe varied between less than 1.5 DID in NL, NO, SE, SK and DK to more than 3.5 DID in FI and FR. HC use varied between five (SK) and 17% (FI) of total consumption in the different European countries. The median (range) proportion of use in 2001 of penicillins (PEN), cephalosporins (CEP), carbapenems (CAR), glycopeptides (GLYC) and quinolones (Q) represented 46% (14–86), 19% (1–35), 1% (<1–3), 1% (0–3) and 9% (1–16) of total HC use, respectively; the median (range) CEP generations represented (from first to fourth): 20% (2–39), 51% (3–87), 25% (8–73) and 1% (0–5) of total CEP use. Consumption was particularly low for PEN (<20%) in BG, FI, and IT and was low for CEP (<7%) in FR and LV. Consumption was high for: CARB (>2%) in ES and SE; for GLYC (>2%) in ES, GR, and IT; for Q (>13%) in ES, FI, SE, SI, and SK.
Conclusions: In HC, a wide variation in total use of PEN, CEP and Q, as well as within the CEP generations were observed. Countries do not seem to cluster in regional supra-national consumption patterns.

O200 Consumption of antibiotics in ambulatory care in Europe: first results of the ESAC retrospective data collection

M. Elseviers, M. Ferech, R. Vander Stichele, H. Goossens
Antwerp, B

Objectives: ESAC (European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2001), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: AC use data were provided by 25 countries; 19 were suitable for international comparison. The remaining five were not comprehensive (AT, LT), or not separable from total data, including HC (BG, CZ, IS, TR).
Results: In 2001, AC use in Europe varied between 10.0 DID (NL) and 32.9 DID (FR). Other high consumers were (in decreasing order) GR, IT, LU, PL, PT, BE and SK, all with a total use >24 DID. During the observation period of 5 years, consumption clearly increased in GR and PL and decreased in BE and ES. High seasonal fluctuations in AC use (mean increase > 30% in quarter one and four compared to quarter two and three) were observed in BE, GR, PL and SI. High seasonal fluctuations were particularly noted in countries with high consumption ($r = 0.77$). In 2001, median (range) proportional use of penicillins (PEN), cephalosporins (CEP), tetracyclines (TET), macrolides (M) and quinolones (Q) was 45 (31–63), 11 (< 1–23), 10 (2–23), 15 (6–23) and 7% (1–14) of total use, respectively; the median (range) proportion of small spectrum PEN, ampicillin/amoxicillin and combination with clavulanic acid was 10 (<1–66), 45 (12–84), and 32% (<1–59) of total PEN use, respectively. Large regional differences could be observed in consumption patterns. Northern European countries (NO, SE, FI, DK, NL, LV) are low consumers using relatively narrow spectrum PEN and TET more extensively and less CEP and Q. Southern European countries (PT, IT, GR, FR) are high consumers using no longer narrow spectrum PEN, but exceptionally high proportions of CEP, M and Q.
Conclusions: Intriguing high variation in AC use in Europe was observed and needs to be related to determinants of use and variation in resistance patterns.

O201 Methodological problems encountered within the ESAC retrospective data collection

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Antwerp, B

Objectives: ESAC (European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, date accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, 2002).
Methods: Data were collected by a pre-established protocol, defining a common format. Additional information was collected by questionnaire: mid-year country population, covered population, nature of ATC/DDD assignment process, data provider characteristics; the number of country bed days (BD) and the BD calculation technique. AC data were to be expressed in DDD/1000 in./day (DID), while HC data in DDD/100 BD.
Results: The following methodological problems were encountered:

- Data originate from disparate sources (sales/reimbursement, census/sample data).
- Bias is possible from extrapolation from sample data or incomplete census data.
- The ATC/DDD assignment process may not be streamlined; most countries stated to have used ATC/DDD 2002 versions, but we observed nonstandardized assignment of DDDs for combinations and substances without official DDD and failure to shift G04A (urinary antiseptics) into J01.
- The number of BDs is a problematic denominator; it proved difficult to reliably and timely estimate BDs and to determine the proportion of long-term rehabilitation or psychiatric BDs.
- The mix between AC/HC is varying, as data from nursing homes, day care centers and policlinic prescribers are differently assigned to AC or HC.
- In reimbursement data, bias in comprehensiveness is possible in Austria, Lithuania, Spain, and Portugal, either because of OTC sales or peculiarities in the reimbursement system.
- In sales data, parallel export or import needs to be determined, as it may distort the validity of the population exposure estimate (e.g. Greece).

Conclusions: Due to uncertain reliability of BD data, their application as a comparable denominator at the international level is jeopardized; hence, we decided to express national HC use data in DID, as in AC. In the upcoming prospective ESAC data collection, it will be possible to adequately deal with most of the methodological problems, encountered in the retrospective data collection.

O202 Data collection performance in the European surveillance of antibiotic consumption: results for the 1997–2001 retrospective data collection

R. Vander Stichele, M. Ferech, M. Elseviers, H. Goossens
Antwerp, B

Objectives: ESAC (European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, date accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002).
Methods: Data were collected according to a pre-established protocol, defining a common format. Additional information was collected by questionnaire: mid-year country population, covered population, nature of the ATC/DDD assignment process, data provider characteristics, and country bed days.
Results: Data from 26 countries were delivered. Two current EU countries were yet missing (Germany and Ireland) and two first wave EU countries (Cyprus, Estonia). In addition, data from Bulgaria, Croatia, Iceland, Norway and Turkey were available. Two countries could only deliver aggregated total data (Iceland, Turkey), two countries only AC data (Austria, England), one country only HC data (Malta), two countries only total and HC data (Bulgaria, Czech Republic), and 19 countries were able to deliver both AC/HC data separately. Hence, AC data were available from 25 countries and HC data from 22 countries. AC data were reimbursement data in 11 countries; in the remaining 14 countries, sales data were provided, collected from companies in two (France, Greece), from wholesalers in nine (Bulgaria, Croatia, Finland, Iceland, Latvia, Norway, Poland, Turkey) and from community pharmacists in three countries (Denmark, the Netherlands, Sweden); in four countries (Austria, Lithuania, the Netherlands, Poland), sample (not census) data were collected. In all but one country (Belgium), HC data were sales data (as hospitals are budgeted in most countries). Nine countries (Belgium, Denmark, England, Finland, Greece, the Netherlands, Poland, Slovenia, and Sweden) were able to deliver quarterly AC data for all 5 years.
Conclusions: For the first time, a credible alternative to industry sources has been established for the collection of internationally comparable data on antibiotic consumption in Europe, based on cooperation between regulatory authorities, scientific organizations, health insurers, and professional organizations.

O203 The impact of cycling antibiotics in a surgical ICU: the Cyclone Trial

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Objective: This study was performed to examine the impact of rotating different antibiotic classes, quinolone and beta-lactams, on microbiologic resistance rates, during fixed time intervals, on a Surgical Intensive Care Unit (SICU).
Methods: Between February 2001 and October 2002, antibiotic policy was rotated in five periods of 4 months. The first cycle levofloxacin was the empiric antibiotic of choice, followed by cefpirome in the second cycle. The third cycle levofloxacin was reintroduced and replaced by piperacillin/tazobactam in the fourth cycle. During the last cycle physicians used the hospital guidelines, with no predominant antibiotic of choice. Sputa and rectal cultures were taken on admission, during their stay and on discharge from the SICU. All cultures and susceptibility patterns were analyzed according to standard microbiological procedures. Resistant strains were stored, for genotyping at a later stage.
Results: Four hundred and twenty-six patients were admitted resulting in 3655 unique isolates. Cycle 1 showed a statistically significant increase in resistance to ciprofloxacin (RR 0.3; CI 0.2–0.5) and a decrease in piperacillin/tazobactam resistance (RR 3.3; CI 1.7–6.5). During the second cycle there was a statistically significant decrease in quinolone resistance and an increase to the beta-lactams. After reintroduction of levofloxacin in Cycle 3 there was again an increase in quinolone resistance (RR 0.4; CI 0.3–0.6). Cycle 4 showed increased resistance to all study antibiotics. In the final period (Cycle 5), resistance levels to all study antibiotics decreased.
Conclusion: Our study shows that changing antibiotic policy has impact on microbiologic resistance patterns. Restriction of levofloxacin showed a decrease in quinolone resistance in Cycle 2. However, an increase was seen again after reintroduction of levofloxacin, with a possible prolonged effect during Cycle 4. This could imply that levofloxacin should have been reintroduced after a longer interval, or that, due to the chromosomal aspect of resistance, quinolones might not be the appropriate choice in cycling. Impact of other factors such as, influx/efflux, cross-contamination, choice of antibiotic pressure and mutation rates still remain unclear. Possibly, quantifying these factors with modeling can give us better insight in which antibiotics to use and what we can expect.

O204 Adherence to local hospital guidelines for surgical antimicrobial prophylaxis. A multicenter audit in Dutch hospitals

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Objective: To study the adherence to local hospital guidelines for antimicrobial prophylaxis in surgery and explore reasons for nonadherence.
Methods: Prospective multicenter audit of elective procedures, without prior suspicion of infection, in 13 Dutch hospitals. Antibiotics prescribed were compared with the local hospital guideline for antibiotic choice, duration of

prophylaxis, dose, dosing interval and timing of the first dose by reviewing medical records, anesthesia records, nurses' reports and medication charts.
Results: Between January 2000 and January 2001, 1763 procedures were studied. Antibiotic choice, duration, dose, dosing interval and timing of the first dose were concordant with the hospital guideline in 90, 83, 89, 43 and 50%, respectively. The most important barriers to local guideline adherence were lack of awareness due to ineffective distribution of the most recent version of the guidelines, lack of agreement of surgeons with the local hospital guidelines, and environmental factors such as organizational constraints in the surgical suite and in the ward.
Conclusion: This study shows that the adherence to local hospital guidelines for surgical prophylaxis in the Netherlands is generally good. However, especially adherence to guidelines on dosing interval and timing needs improvement. When improving the quality of antimicrobial prophylaxis in surgery, efforts should be put in developing guidelines acceptable to surgeons, in adequate distribution of the guidelines and facilitating logistics. Audits of surgical prophylaxis may help hospitals to identify barriers to guideline adherence.

O205 Antibiotic consumption and public attitude: questions and answers

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Objectives: Efforts to reduce antibiotic consumption both in the hospital and the community have been globally linked to the combat against antimicrobial resistance. Public attitude components are essential in guiding the development of antibiotic policy programs and campaigns in the community.
Methods: A survey to evaluate public opinion and beliefs about antibiotic use was conducted in Greece – a country with antibiotic overconsumption and increased resistance rates – through a mailed questionnaire. Three thousand 41 (3041) completed forms were collected out of 5500 dispensed (55%). Seventy-two per cent (72%) were from the greater area of Attica (more than half the country's population) and all social, economic, education levels and occupations were represented.
Results: Male to female ratio was 1 : 1.17 and 80% of the responders were 16–44 years of age. Although the majority (80–88%) knew that antibiotic use can lead to resistance and ineffectiveness of antibiotics and that antibiotics are not innocent drugs to be taken without prescription (72%), 35–65% of responders believed that antibiotics can have antiviral and defervescent effects and offer cure against common cold. Seventy-five percent (75%) had a course of antibiotics during the previous 6 months and among them 40% for symptoms of a viral illness. Of interest is that 85–90% expressed that they consult a doctor to have an antibiotic and that 20% of the population surveyed had some kind of side-effects from antibiotics. Misconceptions and misuse beliefs were greater among the older (>75 years), the younger (<24 years) and those living in the province.
Conclusions: In Greece, antibiotic policy campaigns should aim at increased awareness among consumers about the ineffectiveness of antibiotics against viral illnesses and at improving prescribing habits among primary care physicians.

O206 The use of overnight diagnostic tests to guide antibiotic prescription in general practice

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Objectives: Antibiotic prescribing is a target for control in most countries in both primary and secondary care. In the Grampian region of Scotland, an accelerated bacteriological laboratory examination (ABLE) service has been made available within primary care over the last 24 months in a bid to reduce antibiotic prescribing. This service and its impact on antibiotic prescribing are now described.
Methods: As part of the ABLE service, there is a twice-daily delivery of samples from general practice to the laboratory backed up by the availability of overnight culture and sensitivity results reported either electronically or by telephone. Antibiotic prescribing data for the region were collated and supplied by the National Reimbursement scheme. Laboratory data were extracted from the laboratory information system.
Results: During 1997 there were 88 antibacterial items prescribed per 100 patients in the Grampian region compared to 84 for the whole of Scotland. In 2001, there were 65 antibacterial items prescribed per 100 patient in Grampian and 70 for the whole of Scotland. The majority of general practices make use of the ABLE scheme to some extent. In 2001 the general practices which made high use (greater than 100 samples per year) of the ABLE system ($n = 9$) prescribed 56 antibacterials per 100 patients whereas practices which did not take part in the scheme ($n = 21$) prescribed 68.5 antibacterials per 100 patients. During 2001, 2485 samples were received for processing by the ABLE route. Most common samples were urines (46.8%), throat swabs (25.2%) and sputum samples (10.9%).
Conclusions: Use of the ABLE scheme is associated with a reduced number of antibiotic prescriptions and may be responsible for greater reduction in prescriptions in the Grampian region compared with the rest of Scotland.

O207 Comparison of antimicrobial prescribing in a regional infection unit and two medical admission units

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Objectives: To assess the quality of recording of sepsis markers prior to the commencement of antmicrobial therapy as well as the adherence to local antimicrobial guidelines with regard to the agent used and route of administration. To compare this with data gathered in a Regional Infection Unit.
Methods: Data was gathered in a Regional Infection Unit (IU) as a pilot for the main study in Medical Admissions Units (MAUs) across Scotland, data from the first 349 patients in two centers as part of the main study is shown. Data regarding sepsis markers, antimicrobial agent and route used as well as the grade of doctor prescribing them was recorded. In addition it was recorded whether blood for culture had been obtained prior to commencing antimicrobials. All clinical data was recorded from the admission medical notes within 24 h of admission to hospital.
Results: Data was collected as part of the main study from two MAUs, this followed a pilot study including data from an IU. In the main study the mean age was 68.5 years with a male to female ratio of 55 : 45. With regard to markers of sepsis: 55 patients (16%) had a fever, 13% were hypothermic and 15% did not have their temperature recorded. Thirty-nine percent had a HR > 100/min and 3% did not have their HR recorded. Respiratory rate was >30 in 7% but not recorded in 55%. White blood cell count was raised (>10) in 52% and not recorded in 15%. Regarding compliance with local guidelines, the choice of antimicrobial was in accordance in 51% and the route compliant in 46%. This is markedly at odds with data from the IU were these figures were 86 and 92%, respectively. Blood cultures were taken in 96% of patients in the IU but only 27% in the MAUs, in only 12 cases (3.5%) were these positive. In four cases the antimicrobial was changed as a result of the blood culture isolate and in all four of these the initial choice of antimicrobial was out with the local policy.
Conclusion: Recording of sepsis markers in the admission notes of patients commenced on antimicrobial therapy is poor particularly with respect to respiratory rate. Adherence to antimicrobial guidelines is poor in MAUs in terms of choice and route of administration, but much better in the IU. Culture of appropriate specimens was very poor in the MAUs.

Diagnostics: from gene to machine

O208 Multiplex real-time PCR detection of respiratory viral targets

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Objectives: To develop two multiplex real-time PCR reactions for diagnosis of influenza virus A (FA), influenza virus B (FB), respiratory synctial virus (RSV), parainfluenza viruses 1 (PIV1), 2 (PIV2) and 3 (PIV3) and to assess for use in the diagnostic setting.
Methods: Monoplex real-time PCR assays using molecular beacons as probes were developed for each virus. The molecular beacons were labeled with FAM (FA/PIV3), HEX (PIV1), Texas RED (FB/PIV2) and RSV (Cy5) so that discrimination in a single tube could be determined in one multiplex for FA, FB and RSV and in the other multiplex for PIV1, PIV2 and PIV3. Sensitivity for the assays was obtained by comparison with viral culture by 10-fold serial dilutions of the six different viruses to give a 50% tissue culture infective dose (TCID50). Specificity was obtained by testing each reaction with a range of respiratory viral pathogens. Clinical utility of the assays was assessed by performing viral culture on 220 respiratory samples and comparing to PCR. Culture was performed in shell vials of HEL, LLCK2 and HEp2. Immunofloresence (IF) was performed at 3 days and to confirm any cytopathic effect (Dako, Imagen). The nucleic acids were extracted from stored samples using the Magnapure (Roche) and run in the two multiplex reactions.
Results: The same limit of sensitivity was seen in the monoplex reactions and multiplex reactions, 0.01 TCID50 for FA, 0.001 TCID50 for FB, 0.01 TCID50 for RSV, 0.01 TCID50 for PIV1, 0.001 TCID50 for PIV2 and 0.1 TCID50 for PIV3. The specificity of both the monoplex and multiplex assays were also the same and no other viral respiratory pathogens were detected. In the clinical testing 15 (7%) (two FA, one FB, nine RSV, one PIV1, two PIV3) were positive by cell culture for one of these six pathogens. By multiplex PCR 29 (13%) (two FA, one FB, 15 RSV, five PIV1, six PIV3) were found positive. Nine of the samples positive only by multiplex PCR were from patients with another sample culture positive. Using this real-time PCR methodology results for 30 samples could be obtained in 5 h, including extraction.
Conclusions: The two multiplex real-time PCR for FA, FB, RSV, PIV1, PIV2, PIV3 provides sensitive and specific diagnosis for these respiratory viral pathogens, which are often investigated by direct IF as rapid results are important. This assay provides a sensitive and rapid method and is suitable for diagnostic use and may improve patient management and diagnosis.

O209 Evaluation of a new quantitative ELISA for *Chlamydia pneumoniae* serology in comparison to the microimmunofluorescence test

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Objectives: We have previously shown (JCM 2002, 40 : 1603–9) a high variation of 11 different commercial assays for *C. pneumoniae* serology with regard to sensitivity and specificity using sera from healthy donors. This study showed that the microimmunoflorescence test (MIF) still represents the gold standard although having the disadvantage of subjective evaluation and laborious performance. We extended this approach now to clinical samples and focused on the evaluation of a new quantitative ELISA in comparison to a reference MIF deduced from our previous study.
Methods: Panel 1, 80 serum samples, collected from *pneumoniae* patients and panel 2, 50 serum samples selected with low IgG in MIF from COPD, upper respiratory tract and *pneumoniae* patients were analyzed for anti-*C. pneumoniae* IgG and IgA by the SeroFIA MIF (Savyon) and by SeroCP Quant (Savyon), a quantitative ELISA which allows to define antibody titers comparable to MIF. All MIF were performed and analyzed blindly by the same two experienced persons. panel 3 consisted of 80 serum samples from children negative in both MIF and PCR from tonsillae or adenoid tissue.
Results: On the basis of the SeroCP Quant ELISA, the sero-prevalence of anti-*C. pneumoniae* IgG and IgA in panel 1 was 80 and 66%, respectively (MIF IgG: 81% and IgA: 75%). The concordance of the results of the MIF and SeroCP Quant for panel 1 were 94% for determination of IgG and 86% for IgA. The specificity of SeroCP Quant compared to MIF was 97% for IgG and 96% for IgA, the sensitivity was 81% for IgG and 65% for IgA. All samples which were false negative in SeroCP Quant had very low titer in the MIF. Comparison of the antibody titers measured by MIF and SeroCP Quant in panel 1 showed a high agreement (IgG: $r = 0.92$; IgA: $r = 0.84$). The sera in panel 2 were selected from patients with *C. pneumoniae* infection which displayed low IgG levels. For this second panel, the concordance of the MIF results and SeroCP Quant were above 90% for both IgG and IgA. Again, the antibody titers showed a good correlation ($r = 0.8$). All 80 children were negative for IgG and IgA in SeroCP Quant, except for one with a low IgG titer.
Conclusion: The results of this study show that a partially automated ELISA like the SeroCP Quant resulted in sero-prevalence, sensitivity, specificity and titer levels very similar to MIF. The additional advantage of time saving and objective reading argues for the quantitative ELISAs as a new gold standard in serology.

O210 Comparison of mono and multiplex real-time NASBA for the detection of *M. pneumoniae, C. pneumoniae* and *L. pneumophila* in respiratory specimens

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Introduction: The advantage of nucleic acid amplification techniques (NAAT) is their extreme sensitivity and specificity when compared to traditional techniques. A practical shortcoming of the high specificity is the detection of only the infectious agent that is searched for. Multiplex formats should solve this problem. This may however, lead to a decreased sensitivity in comparison to the individual tests, losing part of the advantage of NAAT. Therefore, there is a need for a comparison between mono and multiplex NAAT.
Objectives: The aim of the study was to develop a real-time multiplex NASBA assay for the detection of *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila* in respiratory specimens based on NASBA amplification of 16S rRNA target sequences using the NucliSens Basic Kit® (bioMérieux).
Methods: Oligonucleotide primers were derived from the *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila* 16S rRNA. The assay was developed using the NucliSens Basic Kit. For real-time detection, molecular beacons were used. Specificity was established on a panel of bacterial strains. The analytical sensitivity of the assay was determined by testing dilutions of a *M. pneumoniae* and *C. pneumoniae* reference strain, and different *L. pneumophila* serotypes and Legionella species in lysisbuffer, or added to pools of respiratory specimens. Subsequently, a limited number of *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila* positive and negative clinical specimens were analyzed. The results obtained with the multiplex NASBA were compared to the results obtained with the mono real time assays.
Results: Specific detection of the 16S rRNA-derived amplicons was achieved by each method. The sensitivity of the multiplex assay was comparable to the sensitivity of the mono-real time assays although the multiplex assay seems to be a little less sensitive than the mono assays: 16/17 of *M. pneumoniae* PCR positive specimens, 2/2 of *C. pneumoniae* PCR positive specimens and 5/6 of *L. pneumophila* positive specimens were positive by multiplex NASBA, whereas all 100 PCR negative specimens were confirmed by multiplex NASBA.
Conclusions: The real-time multiplex NASBA could become a fast, useful and userfriendly diagnostic tool for the detection of *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila* in respiratory specimens. Further evaluation on larger numbers of clinical specimens is necessary.

O211 Molecular panels may help define the etiology of acute viral gastroenteric syndromes

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Objectives: Several viruses, including enteroviruses, rotaviruses, adenoviruses, noroviruses and astroviruses, may be involved in acute viral

gastroenteritis; however, a high proportion of diseases remains undiagnosed, due to the lack of appropriate laboratory methods. Since virus cultivation is not applicable to most enterotropic viruses, we used molecular methods to investigate virus prevalence in fecal specimens of patients with acute nonepidemic gastroenteritis (ANEG).
Methods: Retrospective evaluation was carried out on fecal specimens sent to the laboratory in the period December 2000–September 2002, stored at −80°C. Nucleic acids were extracted by the Boom method. Primer sets to be used in RT-PCR or PCR were deducted from literature. Nested sets of primers were used for enteroviruses, HAV, HEV, and adenoviruses. Amplicons were visualized by ethydium bromide staining. Virus identification and genotype assignment were obtained by RFLP and/or sequence analysis of PCR products.
Results: The analyzed samples were 103. Based on the specific medical request accompanying each specimen, only 15 cases (14.6%) would have been correctly diagnosed. When applying the entire panel of PCR, 37 samples (35.9%) resulted positive to one or more viruses: 10 samples (9.7%) gave positive results to enteroviruses; rotaviruses were documented in 12 cases (11.6%); astroviruses and Norwalk virus were found in four and five cases (3.9 and 4.8%), respectively. Adenoviruses were found in 10 cases (9.7%); HAV was found in two cases (1.9%); no case was HEV-positive. Among positive samples, multiple viral genomes were detected in five cases (4.8%), involving rotavirus + adenovirus (one case), enterovirus + rotavirus (two cases), rotavirus + astrovirus (one case), rotavirus + adenovirus + enterovirus (one case).
Conclusion: The results indicate high virus prevalence in feces from ANEG patients, involving most frequently rotaviruses, adenoviruses and enteroviruses. The concomitant release of multiple viruses is not rare. Based on the specific medical request, only less that half of cases would have been diagnosed as virus-associated, while the application of the entire panel substantially amplified the rate of virus detection. Our data indicate the urgent need for establishment of sensitive and specific molecular diagnostic panels able to concomitantly investigate the presence of enterotropic viruses, to be applied in the screening of both sporadic and epidemic forms.

O212 Repetitive element sequence-based PCR using Intergenic Dyad Sequences: a new typing method for nonencapsulated *Hemophilus* strains

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Objectives: Our aim was to develop a repetitive element sequence-based PCR (rep-PCR) assay as a typing method for nonencapsulated *Haemophilus* strains using a primer based on short repeated elements called Intergenic Dyad Sequences (IDSs), which have been described in several *Haemophilus* genomes and only in two other bacterial genera.
Methods: This study included 90 *Haemophilus* strains: 66 nontypeable clinical isolates of *Haemophilus influenzae* (NTHi) from genital and respiratory tract infections, four reference strains, and 20 genital strains belonging to a cryptic genospecies of *Haemophilus*. IDS-PCR was carried out in high stringency conditions (2.5 mM $MgCl_2$ and annealing temperature of 50°C) with Pfu Turbo DNA polymerase. The resulting patterns were visualized by ethidium bromide staining, photographed, and compared with the help of a computer program. Statistical analysis: (Chi-square test and two-tailed Fisher's exact test) and determination of the discrimination index (D) were realized.
Results: The IDS-PCR technique was rapid, easy to perform and reproducible, with a high discriminatory capacity. The 70 NTHi strains were extremely diverse, yielding 66 different banding patterns ($D > 0.99$). Epidemiologically related strains gave similar or identical fingerprints and all of the unrelated strains except two showed different patterns. For the 20 genital strains usually identified as being biotype IV NTHi and belonging to a cryptic genospecies of *Haemophilus* with a remarkable genetic homogeneity, four bands were significantly present and six bands were significantly absent from the fingerprints ($P < 0.01$). The 20 strains were clustered in 11 closely related profiles ($D = 0.91$). This method improved the differentiation previously obtained within this species by ribotyping and multilocus enzyme electrophoresis.
Conclusion: These results suggest that IDS-PCR is a reliable and discriminatory method for the typing of NTHi strains and can be used to distinguish cryptic genital strains from NTHi strains.

O213 Molecular identification of eight bacteria involved in bacterial meningitis by Pyrosequencing™ technology

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Objectives: Bacterial meningitidis is a serious infection caused by a number of bacterial species. Untreated, the infection can lead to permanent debilitation or death. We have developed a molecular method that can identify and differentiate between the eight species most commonly the cause of bacterial meningitis: *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Enterobacter cloacae*.
Methods: The method presented here is a single PCR amplification of a region within the 16S rRNA gene followed by a DNA sequencing step using Pyrosequencing technology. The two sequencing primers (MenDf and MenFr) used in the DNA analysis were chosen to target a conserved region followed by a highly variable DNA sequences of the 16S gene.
Results: A total of 78 strains including reference strains were used in optimization of the method. In order to measure the validity of the test, the first base that differentiates one strain from the other eight was inspected in the sequencing results. In this way, 97.4% of the tested bacterial strains were successfully typed using the sequencing primers MenDf and 98.7% by MenFr. Experiments to determine the sensitivity of the method was performed both using dilutions of DNA templates, as well as on dilutions of bacteria added before DNA preparation. The sensitivity and the accuracy of the method will be discussed.
Conclusions: The method presented in this study is a DNA sequencing method for rapid and accurate identification of the eight most common bacteria causing bacterial meningitis. Added together, the two obtained DNA sequencing strings (each of about 30 bases) carried enough discriminatory power for proper species identification of the bacteria involved in this disease.

O214 Modeling the combined predictive value of ELISA and Western blot for Lyme disease in a Danish population with a Bayesian network

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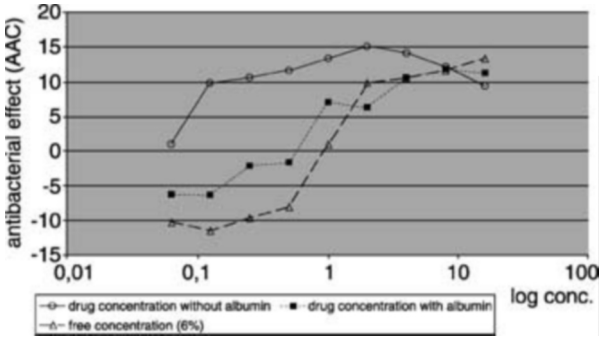
Objectives: To asses the specificity and predictive value of the routine ELISA test by measuring the prevalence of antibodies in the healthy adult population (blood donor samples), and to make an assessment of the added value of a 'confirmatory' Western blot in a theoretical model.
Methods: IgM and IgG serology was performed with IDEIA™ *Borrelia burgdorferi* IgG and IgM (DAKO); Western blot was performed with Lyme Blot IgG and IgM (DAKO) on the positive samples. Modeling of a Bayesian network using a graphical development software (http://www.hugin.dk). The Bayesian network models the probability of active Lyme disease considering the test results in the context of other variables such as false positives, previous Lyme disease.
Results: In 382 blood donors 6 (approximately 2%) were ELISA IgG positive, all six were also Western blot positive. Seven were ELISA IgM positive, one of these only was Western blot IgM positive. None of these ELISA positive had a history of Lyme disease. However, eight other ELISA IgM and IgG negative donors had been treated for Lyme disease.

- As an example the prediction of the model in a patient population suspected of Lyme arthritis the pretest probability of disease was set at 1%.
- The predictive probabilities of Lyme disease for IgM positive/IgG negative does not differ significantly from the pretest probability.
- The predictive probabilities of Lyme disease for IgG positive/IgM negative ranged from 14% in the ELISA IgG positive to 3% in the Western blot negative.

Conclusions:
(1) If both IgM and IgG is ELISA positive, the probability of Lyme disease is relatively high, and there is no need for Western blot.
(2) If IgG ELISA is positive, a positive Western blot does not help to distinguish between previous and current Lyme disease. If Western blot negative the probability of Lyme disease is somewhat lower.
(3) IgM results are of limited value in the diagnosis of possible Lyme arthritis, because of low sensitivity and specificity. IgM Western blot does not seem change the calculated probability of Lyme disease.

(4) Building and using a Bayesian model may support the understanding of the limitations of Lyme serology. However the final diagnostic decision rests with the clinician based on the total evaluation of the patient.



O215 Evaluation of laboratory diagnostics for diphtheria within European Union member states and associated countries

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Objectives: The DIPNET programme is a diphtheria surveillance network (DG SANCO 122/SID/2001) led by the PHLS with representatives that include the key microbiologists and epidemiologists from 15 member states and nine associated countries. One of the main objectives of DIPNET is to determine criteria for the standardization of protocols for the microbiological and epidemiological surveillance of diphtheria. This project will enable the evaluation of optimal methods and typing strategies and support their dissemination to reference centers worldwide.
Methods: In September 2002, questionnaires were sent to diphtheria laboratories throughout Europe to ascertain details regarding the reference services provided within their country, laboratory diagnostic testing procedures, epidemiological typing studies, availability of culture collections, and methods of population immunity screening. Information regarding current methodologies for biotyping and identification of pathogenic corynebacteria including primary selective media, biotyping, toxigenicity testing, and antibiotic sensitivity testing were requested.
Results: Seventy-one percent of the centers that received the questionnaire were national reference centers for their country, although a further 10% had applied for designation. Ninety percent of the centers provided a service for their entire country and 19% also provided a services for cultures referred from other countries. The number of isolates received, the number of diphtheria cases identified, and the proportion that were associated with travel all varied greatly between countries. The importance placed on diphtheria as a public health priory was perceived differently in the various countries. Only 23% of the countries questioned routinely screened throat swabs for the presence of pathogenic corynebacteria. There was some variability in the methodologies and control strains used for biotyping tests and the Elek test. Thirty-three percent of the centers performed epidemiological typing, the most common was ribotyping. Seventy-six per cent of the centers performed serological testing for diphtheria antitoxin levels.
Conclusions: Standardization of methodologies for reference laboratories is essential, especially for toxigenicity testing and molecular typing. Given the immense public health implications associated with the isolation of a toxigenic diphtheria strain, laboratory diagnostic expertise must be maintained.

O216 Ruling out *Bacillus anthracis*: Evaluation of methodology

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Objectives: A panel of conventional tests is applied for the rapid and reliable identification of *Bacillus anthracis* (BA). During a 12-month period, 199 environmental samples suspected of being contaminated with BA spores were submitted for examination and 72 Bacillus strains (all non-BA) were isolated. These strains were used to validate and optimize conventional and molecular methodology for identification or ruling out of BA.
Methods: Seventy-two non-BA Bacillus strains isolated from environmental samples were studied retrospectively. Morphology and hemolysis types were compared on human, horse and sheep blood agar plates (BAP-hu, BAP-ho, BAP-sh). The presence of a capsule was determined after plating on nutrient agar with 0.7% NaHCO3 and India ink staining. Motility was tested in H_2O and TSB at 0 and 2 h of incubation. Selectivity of PEA (selective for non BA strains) and PLET agar (selective for BA strains) were evaluated. Three PCR assays targeting a 152 bp (chromosomal), a 747 bp (pOX1 plasmid) and a 264 bp (pOX2 plasmid) sequence of BA were used for definitive identification of BA.
Results: Discrepant results of colony morphology (type of hemolysis, Medousa head and ground glass appearance) were detected in 45 (62%) isolates grown on the three different BAPs. Beta-hemolytic colonies were produced by 62, 61 and 51 isolates (86, 85 and 71%) plated on BAP-ho, BAP-hu, BAP-sh, respectively. Motility at 0 h of incubation was detected in 33 (46%) and 45 (63%) isolates tested in H_2O and TSB, respectively ($P < 0.00001$). Incubation of 2 h in TSB revealed 13 (81%) additional motile strains, while 3 strains tested in H_2O lost motility ($P < 0.00128$). Twenty-four isolates grew on PLET agar (specificity 67%) and all except 1 grew on PEA agar (sensitivity 97%). Capsule was produced by 2 isolates (incidence 3%). While plasmid sequences were not detectable by the respective PCR assays (specificity 100%), the chromosomal sequence was amplified in 7 isolates (specificity 90%). Five of these strains were among the 24 that grew on PLET agar ($P = 0.037$).
Conclusions: In order to improve timely identification of BA isolates, plating on BAP-ho or BAP-hu and motility testing after incubation in TSB for 2 h are recommended. Chromosomal DNA PCR demonstrates specificity problems and needs to be complemented with testing for plasmid sequences. Although PLET agar supported the growth of a considerable number of non-BA strains, it is still useful as a screening plate.

O217 Comparative evaluation of the VITEK 2 Advanced Expert System in five UK hospitals

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Objectives: A multicenter study was carried out to evaluate the performance of VITEK® 2 AES in comparison with the routine antibiotic susceptibility methodology of five UK Clinical Microbiology laboratories and with a reference agar dilution method.
Methods: Laboratories tested a collection of 82 strains (nine enterococci, 29 staphylococci, 36 strains of Enterobacteriaceae and eight *Pseudomonas aeruginosa*) selected on the basis of their challenging and characterized resistance mechanisms. Over 5000 MICs were determined with VITEK® 2 in the five centers.
Results: In comparison with the reference MIC method, VITEK® 2 gave an overall essential agreement of 96.5% (enterococci), 96.7% (staphylococci) and 95.5% (Gram negative bacilli). Corresponding category (SIR) agreements with VITEK® 2 were 98.0, 95.8 and 94.4% (overall 95.1%). Using five routine methodologies, category agreement ranges were 92.1–100% (mean 95.3%), 95.7–100% (mean 97.1%) and 89.5–96.5% (mean 93.5%) for the three organism groups with an overall agreement of 95.0%. In contrast to VITEK® 2 AES, routine microbiology laboratories did not attempt to detect resistance mechanisms for every antibiotic studied. VITEK® 2 AES achieved 100% agreement with reference data for resistance mechanisms in enterococci (where applicable, routine methods achieved 73.7, 52.6 and 52.6% agreement); 83.3% agreement with reference data for staphylococci (routine methods achieved 76.7, 66.7, 60.0 and 56.7% agreement) and 68.2% agreement with reference data for Gram negative bacilli (routine methods achieved 68.2, 52.3, 34.1 and 22.7% agreement).
Conclusions: The success of VITEK® 2 AES may be attributable to the use of a heavy inoculum in a broth test, which may account for better expression of resistance mechanisms compared to their expression in agar-based tests.

Current issues in the treatment of respiratory tract infections with antibiotics (Symposium arranged by Pfizer)

S226 Characteristics of antibiotic classes used for treatment of lower respiratory tract infections

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Antibiotics are the mainstay of treatment for community-acquired pneumonia. Current guidelines for their empiric use are based largely on clinical experience and/or their in vitro activity. Treatment options are simplified if an etiologic diagnosis is established or highly likely on the basis of rapid tests, such as Gram staining, antigen detection, or amplification techniques. The selection of antimicrobial agents is based on multiple variables, including the severity of illness, the patient's age and ability to tolerate side-effects, clinical features and comorbidity, prior exposure to antibiotics, the epidemiological setting, and treatment cost, as well as the prevalence of drug resistance among respiratory pathogens. Numerous antimicrobial agents are available for the treatment of lower respiratory tract infections (RTIs), but beta-lactams, macrolides, the azalide azithromycin, and fluoroquinolones are used most frequently. The agents should be compared on a variety of levels: pharmacological properties, adverse effects, drug interactions, dosing regimens, and pharmacoeconomics are of particular importance. The pharmacokinetic and pharmacodynamic properties of individual compounds can be integrated to predict whether the antimicrobial concentration at a particular body site will be effective. Antimicrobial agents that have time-dependent activity should be administered in a manner that optimizes the time for which the serum concentration exceeds the pathogen's minimal inhibitory concentration (MIC). Antibiotics in this category include beta-lactams, erythromycin, clarithromycin, trimethoprim-sulfamethoxazole (TMP-SMX), and clindamycin. By contrast, antimicrobial agents, such as fluoroquinolones, that have concentration-dependent activity should be administered so as to maximize the degree to which the peak serum concentration exceeds the pathogen's MIC. Antibiotics used to treat RTIs should be effective against the most common pathogens. It is increasingly important to consider activity against beta-lactamase-producing organisms, penicillin-resistant *Streptococcus pneumoniae*, and atypical pathogens. The current advantages and weaknesses of beta-lactam/beta-lactamase inhibitor combinations, TMP-SMX, tetracyclines, macrolides, cephalosporins, azalides, and fluoroquinolones will be reviewed. Clinical studies are required to confirm that advantages predicted on the basis of pharmacological arguments translate into real improvements in clinical outcomes. Some of these studies will be discussed.

S227 Atypical pathogens in lower respiratory tract infections

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Milan, I

The term 'atypical pathogens' indicates a number of microorganisms that can cause so-called 'atypical pneumonia' and other respiratory (and probably nonrespiratory) diseases. The most important bacteria included in this group are *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella* species. *M. pneumoniae* infection occurs worldwide without significant seasonal fluctuations, and is both endemic and epidemic. *Legionella* species have been consistently isolated from a variety of manmade water reservoirs during nosocomial and community-acquired outbreaks. Direct inhalation from *Legionella*-contaminated water supply systems is the most likely mode of transmission. Patient isolation is not mandatory, as no evidence of person-to-person transmission has been reported. The incidence of *Legionella* infection, which occurs worldwide, is highly variable ranging from 1 to 27% of cases of community-acquired pneumonia (CAP). The clinical severity of *Legionella* pneumonia ranges from mild respiratory disease to fulminating pneumonia. *C. pneumoniae* has been recognized as a cause of respiratory tract infections and is considered the most common nonviral, intracellular, human respiratory pathogen. *C pneumoniae* accounts for 6 to 20% of cases of CAP, depending on several factors, such as the setting of the population studied, the age group examined, and the diagnostic methods used. The frequency of pneumonia due to atypical pathogens has varied considerably during the past decade. However, these pathogens have become accepted as relatively common causes of pneumonia in both outpatient and inpatient settings, and are often present as coinfections. Studies have shown improved outcomes for CAP patients who received therapeutic regimens effective against atypical respiratory pathogens. As well as their involvement in CAP, there is evidence of a role for Mycoplasma and *C. pneumoniae* in both acute and chronic asthma. Both in vitro and animal model studies suggest that atypical agents may play a role in the pathogenesis of the disease. Preliminary data on *C. pneumoniae* chronic infection in patients with chronic obstructive pulmonary disease indicate this agent as a plausible modulator of the natural history of chronic bronchitis and emphysema. This modulation is thought to be attributable to the toxic effect of *C. pneumoniae* on bronchial epithelial cells, ciliostasis, and the ability of the organism to increase chronic inflammation through pro-inflammatory cytokine production.

S228 Pneumococcal resistance in perspective

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Although rates of resistance among pneumococci are rising for important drug classes such as beta-lactams, macrolides, and fluoroquinolones, a debate remains as to whether there is a corresponding increase in the rate of treatment failure. This controversy may also be viewed as whether microbiologic resistance determinations in vitro are predictive of treatment outcome in vivo. A number of studies have addressed the question of whether clinical outcome is affected by the presence of penicillin resistance in patients with pneumococcal disease. While it is generally accepted that drug-resistant *Streptococcus pneumoniae* (DRSP) meningitis is associated with a poor outcome when penicillin alone is used, treatment failure in DRSP respiratory tract infections has yet to be demonstrated convincingly. Clinical studies of pneumococcal pneumonia and/or bacteremia have assessed the treatment outcome following infection by DRSP compared with the course of infection by drug-susceptible isolates among the same population. Some studies have also evaluated outcome according to whether the patient received appropriate therapy for DRSP. A number of factors might contribute to the observed lack of clinically significant outcome differences between drug-susceptible and drug-resistant pneumococcal infections. A major difference is that many of the drugs used to treat pneumococcal infection achieve high levels of tissue and plasma concentration, while the NCCLS break points for intermediate and full resistance are relatively low. A second factor that may play a role in clinical outcome is the immunomodulatory activity of macrolides. Recent studies of patients with severe pneumonia have demonstrated a beneficial effect of dual therapy with a beta-lactam plus macrolide over monotherapy, usually with a fluoroquinolone. These new data, as well as information on current guidelines for managing community-acquired pneumonia in the setting of potential resistance, will be discussed.

S229 The current role of macrolides in the treatment of CAP

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Macrolides remain an important therapeutic option in the management of community-acquired pneumonia (CAP), being suitable for use either as monotherapy in selected patients without complex disease, or as part of a combination regimen in complicated patient populations. Although some experts have viewed quinolones as the first-choice treatment for CAP, this view is not expressed in the latest American Thoracic Society guidelines, which recommend macrolide monotherapy for both outpatients and inpatients with no cardiopulmonary disease and a low risk of infection with drug-resistant *Streptococcus pneumoniae* (DRSP) or enteric gram-negatives. Azithromycin is more cost-effective than a broader-spectrum regimen in qualifying inpatients, and a macrolide/beta-lactam combination is as effective as quinolone monotherapy for individuals managed anywhere except the intensive care unit. There, patients require either a macrolide or a quinolone, combined with other agents, depending on whether risk factors for infection with *Pseudomonas aeruginosa* are present. In appropriate patients, macrolide monotherapy is not only cost-effective but also represents the most focused therapy possible, avoiding unnecessary broad-spectrum coverage, which could drive future antibiotic resistance. Used in combination with a beta-lactam, macrolide therapy provides coverage of atypical pathogen coinfection, affording reductions in both mortality and length of stay, compared with

beta-lactam monotherapy. A beta-lactam/macrolide combination is also an important, effective alternative to quinolone monotherapy; CAP patients recently treated with a quinolone have an enhanced risk of infection with a quinolone-resistant pneumococcus. Although macrolide-resistant pneumococci are becoming more prevalent, this has rarely led to clinical failure, except in patients incorrectly assigned to macrolide monotherapy. The relatively low rate of clinical failure with this therapy may be due to resistance being commonly mediated by an efflux, and not a ribosomal, mechanism, which produces a level of resistance that can generally be overcome by the excellent penetration of macrolides into respiratory secretions. Ribosomal resistance is more of a potential clinical concern, and patients at risk of infection with DRSP should receive macrolides in combination with selected beta-lactams. Used in this way, macrolides remain a safe and valuable option for the therapy of CAP, both in and out of the hospital.

What can a molecular typing service mean for a clinician (Symposium arranged with ESGEM)

S230 What contribution does molecular typing make to patient care?

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In the management of an individual patient with infection, the identification of a pathogen and confirmation of its anti-ineffective susceptibilities are the major priorities. However, the typing of pathogens has a significant role to play in the management of the individual patient and in the management of infection generally. Clusters of infection are common, including in hospitals, and it is important to try to determine whether these occur by chance, from a common source, or as a result of cross-infection. Typing, especially using molecular methods, is the most useful approach to determining which of these is most likely. Examples include clusters of pseudomonal infection in ICU or the pattern of MRSA infections throughout a hospital. In the case of the individual patient who has a serious bloodstream infection, it can be difficult to distinguish recurrence, i.e. failure to eradicate in the first instance, from re-infection, i.e. a second infection with a different strain. This distinction has significant implications for therapy as recurrence may require a surgical approach as well as antibiotics. Typing of organisms in this setting therefore has the potential to guide more effective management of the individual patient. The challenges for molecular typing are to answer these and other questions, not only reliably, but within a realistic time-frame. In this way, infection may be prevented by earlier recognition of cross-infection and antibiotics better targeted on the basis of a deeper understanding of the pathogenesis.

S231 Molecular typing for infection control: when, how, and how much?

P. T. Tassios
Athens, GR

Everybody knows that successful infection control relies on successful team work. So how can molecular typing, with its increasingly diverse and sophisticated methods, become a truly integrated contributor to infection control?

- When (in which cases? how often?) should molecular typing information be requested regarding a nosocomial infection? When (how fast? how frequently?) should the answers from typing be given?
- How (which methods? in answer to what kind of questions?) can molecular typing information help in the most economical and productive way? How should typing results be interpreted? And how could they be used to assist infection control decisions?
- How much sophistication in molecular typing is useful? How much should clinicians expect from molecular typing data? And how much should they rely on them.

To illustrate and partly answer the above (and other related) questions, examples from the presenter's experience and the published literature will be used – but, more importantly, challenges and contributions from the participating audience are encouraged and will be gratefully taken up.

Epidemiology and management of multidrug-resistant tuberculosis in Europe

S232 DNA typing of *Mycobacterium tuberculosis* isolates for epidemiological research

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Objective: To give an overview of the main achievements of the application of DNA typing of *Mycobacterium tuberculosis* isolates.
Methods: Molecular epidemiology on basis of IS6110 restriction fragment length polymorphism (RFLP) typing.
Results: In the Netherlands, from 1993 on strain typing has been used to support the contact tracing. Before IS6110 RFLP typing results were reported to the regional TB nurses, the relationship between patients was 'expected' in 462 (21%) patients. After cluster feedback on basis of RFLP analysis, epidemiological links were documented in 551 (25%) cases and assumed in 432 (20%) cases. Unfortunately, not all identical DNA fingerprints of *M. tuberculosis* isolates indicate recent transmission. Even with extended interviews not all transmissions can be explained. Recently, a part of the isolates of patients with known and unknown links grouping in clusters on the basis of IS6110 RFLP typing were subjected to VNTR typing. Four of 11 clusters comprising patients with unknown links were re-grouped in smaller clusters on the basis of VNTR typing. Recently, it was found in Ho Chi Minh City, Vietnam, that 25% of the relapses after curative treatment are due to infections caused by new strains. In the Netherlands 29/183 (16%) isolates from patients with reported tuberculosis infection or disease before 1981 were found in recent-transmission clusters presumably reflecting re-infection. With DNA fingerprinting it was found that, in the Netherlands, in the period 1993–2000, 2.4% of the positive *M. tuberculosis* cultures were due to laboratory cross-contamination. Furthermore, it was demonstrated that five patients contracted severe *M. bovis* BCG infections caused by nosocomial transmission in pharmacies. Strain typing in different geographic areas has revealed that the population structure of *M. tuberculosis* differs significantly between settings with a low and a high incidence of tuberculosis. In Asia, the *M. tuberculosis* Beijing genotype is highly prevalent, and this genotype is associated with drug resistance in some areas. Research on the immunopathology caused by strains of different *M. tuberculosis* genotypes in a BALB/c mouse model revealed that Beijing genotype strains are also extremely virulent.
Conclusion: DNA typing of *M. tuberculosis* isolates has greatly facilitated the possibilities to study transmission of tuberculosis at various scales. The new insights into the population structure of this bacterium will contribute to future vaccine development.

S233 Current and future drug treatment for tuberculosis

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Isoniazid and rifampicin are the most important drugs in the treatment of tuberculosis. The effective mycobacterial killing power of isoniazid rapidly renders the patient sputum smear negative and therefore noninfectious. Rifampicin is the best drug at eliminating persistent organisms and therefore enables treatment to be reduced to less than a year. A third drug, pyrazinamide, given in combination with the first two drugs for the initial two months

enables treatment to be reduced to a total of six months. In practice, because of increased drug resistance, a fourth drug, usually ethambutol is also given for the first two months or until sensitivity results are available. Streptomycin can be used as an alternative to ethambutol in severe disease such as tuberculous meningitis. Because of the increasing problem of drug resistance the older second line drugs are still necessary. broadly these fall into two categories, oral drugs such as cycloserine, PAS, thiacetazone and ethionamide and injectables such as amikacin, kanamycin and capreomycin. No new drugs have been developed specifically for tuberculosis since rifampicin, 40 years ago but a number of newer drugs have been found to have activity against *M. tuberculosis*. Of these, the quinilones, ofloxacin and ciprofloxacin have been found to be effective in clinical trials. Clarithromycin, the combination of clavulanic acid and amoxicillin and clofazimine are also effective against *M. tuberculosis*. New approaches using immunomodulation are of limited use. Gene modifaction such as alteration of the expression of isocitrate lyase, important for bacterial resistance in the mouse, may also be of use in the future.

Emerging viral zoonoses

S234 Hantaviruses: from asymptomatic infections in rodents to major human pathogens

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Pathogenic European hantaviruses include Puumala (PUUV) and Saaremaa (SAAV) causing mild HFRS, and Dobrava (DOBV) causing severe HFRS. These infections may be diagnosed using IgG and IgM EIAs based on the use of capture antibodies and recombinant baculovirus-expressed nucleocapsid (N) proteins. The early IgG response is towards N protein. IgG antibodies to glycoproteins (G1 and G2) develop at convalescence and are more type specific. For point-of-care diagnostics, sensitive 5-min IgM-antibody tests were developed. Serological screening of rodents followed by PCR, genetic analysis and virus isolation led to discovery of Tula virus (TULV) from European common voles and Topografov virus (TOPV) from lemmings. TULV can infect humans but the pathogenicity of TULV and TOPV is not known. Phylogenetic analyses show that (i) hantaviruses coevolve with their natural carrier rodents, (ii) genetic variants are clustered according to their geographic origin, (iii) hantaviruses in individual hosts are represented as closely related variants (quasispecies), (iv) host-switching can occur during the evolution of hantaviruses, and that (v) recombination and reassortment can occur within a genotype. The pathogenesis of HFRS is not well understood. Increased capillary permeability is thought to be involved. PUUV infection induces dominant and polyclonal cytotoxic T-lymphocyte responses to the N protein. In cell culture, hantaviruses cause little or no cytopathic effect. PUUV-N interacts with the Fas-mediated apoptosis enhancer Daxx and G1 is apoptotic unless coexpressed with N or the mitochondrial apoptosis inhibitor Bcl-2. Thus, PUUV may regulate apoptosis. A monkey model for PUUV-induced HFRS may elucidate the pathogenetic mechanisms. The severity of NE is associated with HLA B8 DR3 haplotype and correlates with the relative ease of RT-PCR detection of PUUV sequences from patient blood and urine. In contrast, HLA B27 is associated with a mild course of NE. As a late consequence 20% of NE patients develop elevated blood pressure and proteinuria. Some patients develop hypophyseal insufficiency. Studies are in progress to develop antivirals from phage-display peptide libraries and use of recombinant proteins (N protein, and G1 and G2 from separate constructs) as vaccine candidates. When the protective capacity was studied in a bank vole infection model using DNA immunization, best protection was obtained with PUUV-N.

S235 West Nile fever: commotion about a (re-) emerging virus in Europe

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West Nile (WN) virus is an arbovirus from the Flaviviridae family, transmitted by mosquitoes. It was initially isolated from human in Uganda in 1937. Birds are involved in the cycle of transmission of the virus as amplifying hosts. Humans and horses are sensitive to the virus and considered as accidental dead-end hosts. The geographic distribution included Africa, the Middle East, India and Southern Europe. West Nile virus was initially considered as an minor arbovirosis, with no apparent pathogenicity in birds, and usually inducing a non symptomatic or mild flue-like illness in humans. However some cases of encephalitis were reported in the 1950s in Israel, in France in 1962–1963 both in humans and horses, and fatal cases in India in children. Since the last 10 years, several outbreaks with unusual patterns are reported mostly in the Mediterranean basin with fatal encephalitis cases mainly among elderly people: Algeria in 1994, Romania in 1996, Tunisia 1997, Russia (Volgograd) in 1999 and epizootics in horses in Morocco in 1996, Italy 1998 and France in 2000. In 1997–1998, in Israel, WN was identified in migrating storks and domestic birds (geese) with clinical symptoms of encephalitis and paralysis. A large epidemic occurred in 2000. A similar viral strain suddenly emerged in New York during the summer 1999 killing various species of birds along with cases with fatalities in humans. The virus is now well established in the New World and the bird mortality is a key indicator for WN surveillance there. In contrast in Europe no unusual fatalities in birds were noticed during the last outbreaks. In Israel in 2000, several strains of WN virus were cocirculating. Phylogenetic studies have shown two main lineages among the identified WN strains. Strains from lineage I are circulating in Africa, India and are responsible for the outbreaks in Europe and in the Mediterranean basin. Strains from lineage II are circulating only in Sub Saharan Africa and Madagascar. Studies of the genetic susceptibility of the hosts are in progress. The immediate adaptation of WN virus in North America shows the capacities of this arbovirus to disseminate in a continent due to the wide variety of competent mosquito vectors, the possible role of ticks and the movements of birds. The virus may circulate widely among birds and mosquitoes in Europe and a close surveillance may reveal the presence of the virus in different locations. West Nile viral activity was reported in France in 2001 and 2002 in monitored sentinel birds in the Camargue area, as well as in Romania with yearly identified encephalitis cases in humans since 1997. West Nile virus will circulate in Europe in 2003 but the occurrence of outbreaks still remains unpredictable.

Arthropod-borne viral diseases and viral hemorrhagic fever: a threat to Europe?

S236 Molecular biology and pathogenicity of Marburg and Ebola viruses

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Marburg and Ebola viruses which are indigenous to tropical Africa cause fulminant hemorrhagic fever in man and nonhuman primates with mortality rates up to 90%. Outbreaks are rare, but usually attract a lot of public attention because of the dramatic course of the disease. The pathophysiological changes that make these virus infections so devastating are not fully understood, but it appears that vascular leakage, dysregulation of cytokine production, and immune suppression are important mechanisms in pathogenesis. The molecular biology of Marburg and Ebola viruses which together constitute the filovirus family has been analyzed in recent years in some detail. Filoviruses have a nonsegmented negative-stranded RNA genome encoding seven structural proteins: four components of the ribonucleoprotein complex (NP, VP30, VP35, and L), two matrix proteins (VP40 and VP24), and the spike glycoprotein. In addition, there are nonstructural, soluble glycoproteins that are derived from the glycoprotein gene by transcipitional editing and post-translational processing. The role of these proteins in virus replication, pathogenesis and immune protection will be discussed.

S237 Crimean–Congo hemorrhagic fever: a public health risk for countries of the European Union?

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Legnaro, I

Crimean–Congo hemorrhagic fever (CCHF) virus is the etiological agent of a tick-borne zoonosis present in Africa, Asia and eastern Europe which causes human illness with an approximately 30% fatality rate. The virus is a member of the Nairovirus genus of the family Bunyaviridae. The disease is widely distributed in Asia, Africa and eastern Europe. With reference to the European Union it has been reported in Greece, France and Portugal – however, it should be stated that the evidence for France, Portugal and Turkey is based on very limited observations. The virus has been isolated primarily from cattle, sheep, goats, ostriches and hares and antibodies have been detected in a range of wild and domestic vertebrates. Infection can be transmitted by ticks of several genera although experimental and field evidence indicate that the most efficient vectors appear to be members of the genus Hyalomma. In fact, the world distribution of the virus coincides with the distribution of these ticks. The virus causes mild infection with transient viremia in farm and wild animals, while it causes a severe clinical condition in human beings. The disease is a hemorrhagic fever with a fatality of 30%. Clinical symptoms are determined by liver and endothelial damage and impairment of hemostasis. Platelet counts drop dramatically and there is evidence of widespread hemorrhages. Disseminated intravascular coagulopathy occurs and contributes to further tissue damage. The present paper reviews the potential risks of introducing the disease into the European Union through the movement of live animals or of animal products from African and Eastern European countries. In particular, the actions undertaken to prevent the introduction of infected animals or vectors through the importation of ostriches and ostrich products into the EU are discussed.

Issues on screening for *Chlamydia*

S238 Screening programs for *Chlamydia* are effective, and cost-effective

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Background: The introduction of nonculture tests for *Chlamydia trachomatis* in the early 1980s enabled widespread screening of women. In the latter 1980s, the first multiyear regional screening program among women resulted in population prevalences dropping to <0.5 those of initial prevalences. Over time, and with the advent of increasingly sensitive tests, additional community and regional screening programs have demonstrated similar falls in prevalence. That these falls in prevalence are accompanied by lessened rates of pelvic inflammatory disease (PID) was first reported from a controlled trial by Scholes et al. These findings are supported by data from other intervention programs, showing that testing is associated with lessened rates of PID and its complications. That screening programs for young women are cost-effective has been shown in a number of cost-effectiveness studies. The study designs are heterogeneous, and cost-saving threshold prevalences for screening range from a low of about 4% to as high as 14%, with general clustering at about 5–7%, a threshold prevalence below that found in most populations of young, sexually active women. Current dilemmas include how best to use newer, noninvasive *Chlamydia* tests; how to identify women at highest risk; how to detect or treat infected male partners; and, at what prevalence of infection will we no longer recommend screening. Fewer data have examined whether screening young men leads to lessened rates of complications or to lessened rates of infection in women. Using noninvasive testing on urine, limited modeling suggests screening is cost-effective at prevalences at or lower than those of young women, and prevalence surveys largely find prevalences of infection among young males that rival prevalences among young females. The few data of the impact that screening young women has on male prevalences of infection suggest the impact is minimal.
Conclusion: Screening programs for *C. trachomatis* among young women have been successful in both decreasing prevalence of infection and complications of infection. They should be continued, although modifications must be made as test options and prevalences change. There are fewer data addressing the value of screening of young males, although from a clinical and epidemiologic view, such programs 'make sense'.

S239 Issues on screening for *Chlamydia*

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Antwerp, B

Urogenital *Chlamydia trachomatis* (CT) is the most common curable sexually transmitted infection in the developed world, with high prevalence rates in different settings and population groups. CT is a major cause of pelvic inflammatory disease, ectopic pregnancy and infertility. The economic and human costs of managing these complications are considerable. The infection is largely asymptomatic, so early detection strategies are important in prevention and control. However, there are still some gaps in the evidence which limit support for routine screening. Further research on feasibility and cost-effectiveness is needed before large-scale screening programs can be implemented. Knowledge of the natural course of CT infection is limited. The need for screening is dictated by the frequency of serious complications, but estimations are mostly derived from studies in symptomatic women or from case-control studies. There is evidence that the rate of complications in asymptomatic infections is lower than previously thought. This finding affects cost-effectiveness calculations. There is no consensus on who is to be screened. Risk factors for infection vary in time and in different settings, and criteria for selective screening are setting-specific. There is evidence that selective screening of women reduces the incidence of PID. The effectiveness of screening men in reducing complications in women, in reducing transmission, or in improving men's health have not been studied sufficiently. It is not known to what extent screening of selected population or patient groups has impact on CT prevalence in the general population. The development of highly sensitive and specific DNA-amplification tests has made noninvasive testing possible, but these diagnostics should be carefully used in routine clinical practice or in community screening programs. Confirmatory testing is recommended to avoid false positive diagnoses but this is not yet a routine. Furthermore, there are few data to relate a positive amplification test with clinical outcome. Treatment is assumed to be very effective, but microbiological cure has only been studied in the short term. Relapse-or reinfection rates are insufficiently known, but are important to determine screening intervals. Finally, potential harms from screening have not been studied. These include stigmatization, fear for infertility and partner discord, and can greatly affect the quality of peoples' lives.

New experimental systems: studying bacterial pathogenesis in ameba and nematodes (Symposium arranged with the ENSEI)

S240 Analysis of bacterial virulence in an ameba host

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Bacterial virulence is a measure of the ability of a bacterium to cause a disease when confronted to a host organism. Practical and ethical problems limit severely the use of mammalian hosts, and this has led to the development of alternative host models. We have used *Dictyostelium amoebae* to study the complex relationship between bacteria and phagocytic cells. In this simple system we performed genetic analysis to identify virulence genes in *Klebsiella pneumoniae* bacteria, as well as host resistance genes in the ameba host.

S241 *Caenorhabditis elegans* and the study of universal bacterial virulence factors

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For certain pathogens capable of infecting a broad range of organisms, there exist universal virulence factors, necessary for full pathogenicity regardless of the host. This has been most clearly demonstrated by Ausubel and colleagues for the human opportunistic pathogen *Pseudomonas aeruginosa*. As a consequence, one can use nonmammalian model systems, including the nematode worm *Caenorhabditis elegans*, to assay for such virulence factors. A significant number of pathogens of *C. elegans* that provoke a range of diseases, are now known, including the opportunistic human pathogen *Serratia marcescens*. After explaining the practical advantages associated with the use of *C. elegans*, and briefly reviewing previous studies, the results of a screen for *S. marcescens* virulence factors will be presented.

New aspects of anaerobic infections (Symposium arranged with the ESGARAB)

S242 Characterization of anaerobic bacteria using MALDI-TOF-mass spectrometry: a novel approach to characterizing pathogenic microbes

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Changes in global temperature, man's increased travel and a large number of agro-economic practices are some of the factors now believed to be contributing to the emergence and re-emergence of many human infections. Consequently microbiological surveillance is likely to be increased dramatically to cater for these needs thus requiring high-throughput techniques that are simple to perform, reproducible, require minimal sample preparation and can be compiled and interrogated though assembled databases. MALDI-TOF-mass spectrometry in a novel technique that fulfills many of these criteria and is likely to have a major impact in such applications. Only a few bacterial cells are required for analysis and there is virtually no sample preparation. Cells are simply placed on a very small sample well (~1.5 mm diameter) of an inert stainless steel target plate, a minute amount of an organic reagent (referred as a matrix solution) added and left to dry for a few minutes. During this period, the matrix solution cocrystallises with the molecules on the surface of intact cells. The admixture is then placed into the sample port of the MALDI-TOF-MS and the energy of a laser beam directed at it. Part of this energy is transferred to the sample and some molecules are ionized while others are desorbed (hence the acronym MALDI – matrix-assisted, laser desorption/ionization). The ions which are negatively and positively charged travel through a tube within an electrical field to a detector at the opposite end, depending on the mode in which the instrument is set. If for example, the instrument is set in the positive mode, only negative ions will reach the detector. The smallest ions will have the fastest 'flight time' (hence the abbreviation 'TOF, Time of Flight') and will arrive first at the detector and produce an electrical signal which is seen as a peak on the recorder. This provides an accurate measurement of the molecular weight of that ion. As more ions arrive, the output recorder generates a complex profile of the ions present in a sample in less than 2 min These can range from just a few to several hundred thousand Daltons and covers the molecular masses of proteins which are orders of magnitude greater than simple organic compounds. Several factors such as the type of matrix solution, sample preparation, etc. will influence the range of ions seen in the mass spectrum. Based on large numbers of samples, we set up our protocol to collect ions within the range of 500–10 000 Da for comparative analysis of microbes. The microorganisms analyzed represent many diverse branches of the bacterial kingdom but with a strong bias towards human pathogens. For every group studied so far, a unique mass spectrum has been recorded. We are currently assembling a database of these mass spectral fingerprints in a large collaborative study at three centers; Micromass UK Ltd (Waters Cooperation), Manchester Metropolitan University and the PHLS, London. One of the great benefits of this multicentre study is access to bacterial cultures from the National Collection of Type Cultures. This, the oldest such depository in the world (from 1920), contains several thousand well-characterized reference microorganisms that are being used to assemble this core database. The generated database contains the mass spectral profiles of over 2000 species and is now being used to test field isolates from hospitals, etc. It is too early to provide definitive answers on whether field isolates will be sufficiently similar to those present in the database to enable its rapid identification, but early results on approximately 200 isolates show over 80% success. This is expected to improve as the database expands to include more species. Mass spectrometry has been used traditionally for analysis of small molecular weight compounds (*c.* 1000 Da) which often necessitated prior purification or crystallization. The ions in these samples were produced by a powerful electron beam which fragmented the molecule to produce a characteristic mass spectrum. The larger molecular ion enabled an accurate determination of its molecular weight while the fragmentation pattern of the molecule also helped to piece together its chemical structure. Because of the size and share complexity of proteins, this powerful technique remained outside the scope of biomolecules. MALDI-TOF-MS has dramatically changed this and is finding many applications across the fields of biology and medicine. In the application discussed here, viz the rapid identification of microbes, there may be additional benefits relating to the topology of the cell as the data is assembled and analyzed. The bacterial surface of a cell is dynamic and many pathogenic factors e.g. attachment molecules that enable cells to adhere to specific tissues to initiate disease, toxins, antibiotics resistant molecules, cell-signaling molecules, etc. are located here. Since MALDI-TOF-MS produces a mass fingerprint of the surface components of intact cells, this may shed light on the changes in virulence and pathogenicity of certain species and help identify novel epitopes and possible vaccine targets in the process of screening.

S243 Fifty years with anaerobes, or how lucky can one guy be?

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I never heard about anaerobes in college (though a Bacteriology major), nor in medical school, except for a brief introduction to gas gangrene, tetanus and botulism. I didn't have formal training in anaerobic bacteriology so I taught myself, not very well and not very quickly. As a medical student in 1948–1949, I did bacteriologic studies on dog feces under a Department of Surgery protocol. 'Anaerobes' were grown by inoculating stool dilutions on Brewer plates. Facultative anaerobes were not recognized, so everything growing on these plates was called an anaerobe. In 1951, I saw, but did not recognize as such, my first patient with an anaerobic infection; he had putrid empyema with negative culture of the pus. In 1954, I first isolated an anaerobe, subcultured it, and identified it as a 'Bacteroides'. My mentor, Dr William Hewitt, encouraged me to isolate 100 anaerobes and do antimicrobial susceptibility studies on them; this almost scared me out of the field. Reviewing the literature was exciting (but difficult, because this antedated personal computers). I was amazed by how much the French and German scientists knew in the late 1800 s. I finally met other people interested in and knowledgeable about anaerobes and learned a great deal from them. Included among these were such masters as Louis DS. Smith Ron Gibbons, Peg Holdeman and Ed Moore, Trevor Willis, Andre Prevot, and Ivan Hall. I had the pleasure of several extended discussions with Louis Smith, and profitable and fun visits to the laboratories of Robert Hungate, the VPI group, and Bud Dowell at CDC. I began clinical studies on anaerobic infections in the mid-1950s and was amazed at how frequently anaerobes were involved in a wide variety of processes. I also began bowel flora studies about this time, although I did do one study as a medical student that was published in 1951 – my very first paper. At first, I used line gas, but fortunately our local gas supply was not very toxic to anaerobes. Eventually, we used cylinders of known gases, an anaerobic chamber, and a gas chromatograph. Later, we did cellular fatty acid determinations. Finally, we are doing molecular work, with G + C determinations, 16S rRNA sequencing, DNA–DNA hybridization, real-time PCR, etc. One of our major projects currently is evaluating the role of bowel flora in autism.

In vitro activity of various antibiotic compounds

O244 Comparison of in vitro activity of moxifloxacin and ciprofloxacin against clinical isolates of *Acinetobacter baumannii* from hospitals in the UK

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Objectives: To compare the in-vitro activity and mechanisms of resistance of moxifloxacin and ciprofloxacin against clinical isolates of *Acinetobacter baumannii* from UK hospitals.
Methods: MICs of ciprofloxacin and moxifloxacin were determined by *E*-test for 226 nosocomial isolates of *A. baumannii*. PCR was used to screen for chromosomal mutations in the *gyrA* and *parC* genes. Isolates resistant to ciprofloxacin and sensitive to moxifloxacin were examined for the ability to generate spontaneous moxifloxacin-resistant mutants in the presence of increasing concentrations of moxifloxacin.
Results: Based on BSAC guidelines, 49.1% (111) of isolates were resistant to ciprofloxacin, and 39.4% (89) showed moxifloxacin resistance. MICs of moxifloxacin were generally lower than those of ciprofloxacin, and no isolates were moxifloxacin-resistant and ciprofloxacin-sensitive. A *gyrA* mutation at Ser-83 was found in all ciprofloxacin-resistant isolates. Single mutations in both the *gyrA* and *parC* genes at codons Ser-83 and Ser-80, respectively, were located in all but two of the ciprofloxacin and moxifloxacin-resistant isolates. Ciprofloxacin-resistant, moxifloxacin-sensitive isolates generated spontaneous moxifloxacin-resistant mutants at up to 8× their initial MIC. However, none of the mutants generated displayed high-level moxifloxacin resistance (>8 mg/L). The mechanism of resistance to moxifloxacin in these mutants appeared to be either altered permeability or efflux pump-mediated as none of the mutants possessed the *parC* mutation associated with moxifloxacin resistance. Furthermore, the moxifloxacin resistance generated was highly unstable and was lost if mutants were subcultured onto agar lacking moxifloxacin.
Conclusions: Moxifloxacin demonstrated greater activity than ciprofloxacin against clinical *A. baumannii* isolates in vitro. A mutation in the *gyrA* gene conferred resistance to ciprofloxacin in *A. baumannii*. Mutations in *gyrA* and *parC* genes were required for significant resistance to moxifloxacin, although altered permeability and efflux pumps may also be involved. Moxifloxacin-resistant mutants were not readily selected in vitro at moxifloxacin concentrations found in vivo. Moxifloxacin retained potentially useful activity against some multidrug resistant *A. baumannii* isolates and could be a useful therapeutic alternative to ciprofloxacin for the treatment of *A. baumannii* infections.

O245 In vitro activity of antibiotic combinations against multiresistant Gram-negative isolates from cystic fibrosis patients

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Objective: Multi-resistant Gram-negative isolates from cystic fibrosis (CF) patients were sent from CF centers around Scotland to assess best therapeutic antibiotic combinations. A league table of appropriate combinations was constructed.
Methods: Isolates were collected between February 1999 and March 2002. Ninety-four *Pseudomonas aeruginosa*, 31 *Burkholderia cepacia*, 22 *Stenotrophomonas maltophilia* and five *Achromobacter xylosoxidans* were tested to a variety of antibiotic combinations using the *E*-test method with 24 h incubation. Fractional inhibitory concentration indices were calculated. Six hundred and ninety-three antibiotic combinations were tested including: 289 × aminoglycoside/beta-lactam, 175 × ciprofloxacin (CIP)/beta-lactam, 51 × CIP/nonbeta lactam, 42 × colistin (COL)/nonbeta lactam, 76 × ceftazidime (CAZ)/nonbeta lactam and 60 × minocycline (MIN)/CAZ or cotrimoxazole (COTRIM). Each combination was tested on 10–70 bacterial isolates.
Results: Six percent combinations were synergistic, 30% additive, 63% indifferent and 1% antagonistic.
Top three synergistic combinations were CAZ/amikacin (AMIK) (24%), CAZ/gentamicin (GENT) (18%) and CAZ/MIN (15%). The following combinations showed no synergy: amikacin/imipenem, tobramycin (TOB)/Meropenem (MERO), CIP/TOB, COL/TOB, COL/CIP, MERO/CIP, CO-TRIM/CIP and COTRIM/CHLOR. The most frequently additive combinations were TOB/MERO (48%), CAZ/TOB (46%), CIP/MERO (45%) and CAZ/CHLOR (44%). CIP/TOB (8%) CIP/COL (8%) and CAZ/COTRIM (9%) showed the least additive effect. CIP/TOB (92%) and CIP/COL (91%) showed the most indifference. One hundred and sixty-nine out of 460 combinations were synergistic or additive against *P. aeruginosa*, 47/150 against B. cepacia, 20/45 against *S. maltophilia* and 11/23 against *A. xylosoxidans*.
Conclusion: Overall, very little antagonism was demonstrated (1.5%) for any combination although the level of synergy was disappointing (6.1%) Even though most interactions were either additive or indifferent, results were considered clinically useful.

O246 Determination of antimicrobial activity of Quinupristin/Dalfopristin and linezolid against Enterococci and Staphylococci using disk diffusion method

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Objectives: To determine the in vitro activity of Quinupristin/Dalfopristin (Q/D) and Linezolid (LZD) against recent clinical isolates of enterococci and staphylococci.
Methods: A hundred and seventy isolates of *Staphylococcus aureus* (87 methicillin sensitive and 83 methicillin resistant), 76 isolates of enterococci (7 vancomycin resistant) were tested for susceptibility against Q/D (15 μg) and LZD (30 μg). Enterococci were typed to the species level by BBL Crystal System®.
The strains were isolated from wounds, urine, blood, respiratory tract infections. The disk diffusion method was performed according to the National Committee for Clinical Laboratory Standards (NCCLS), 2002. *Staphylococcus aureus* ATCC 25923 was used for quality control.
Results: Of the 76 enterococci isolates, seven were vancomycin resistant (six *Enterococus fecalis*, one *Enterococcus fecium*). The disribution of remaning vancomycin sensitive strains were as follows: 56 *E. fecalis* (81%), nine *E. fecium* (13%), one *E. casseliflavus* (1.5%), one *E. raffinosus* (1.5%),1 *E. avium* (1.5%), one *E. durans* (1.5%). All enterococci (including the vancomycin resistant isolates) were sensitive to LZD. The results of disk diffusion test performed by using Q/D and LZD are shown in Table 1.

Table 1 Antimicrobial susceptibility of Q/D and LZD against Staphylococci and enterococci[a]

	Quinupristin/Dalfopristin (Q/D)[b]			Linezolid (LZD)		
	Resistant (R)	Inter-mediate (I)	Sensitive (S)	Resistant (R)	Inter-mediate (I)	Sensitive (S)
S. aureus						
MRSA ($n=83$)	11 (13.3%)	0	72 (86.7%)	4 (4.9%)	–	79 (95.1%)
MSSA ($n=87$)	9 (10.3%)	0	78 (89.7%)	1 (1.1%)	–	86 (98.9%)
Enterococcus spp.						
E. faecalis ($n=56$)	–	–	–	0	0	56 (100%)
E. faecium ($n=9$)	0	2 (22.3%)	7 (77.7%)	0	0	9 (100%)
Enterococcus spp. ($n=4$)[c]	3 (75%)	0	1 (25%)	0	0	4 (100%)
Vancomycin resistant enterococci ($n=7$)						
E. faecalis ($n=6$)	–	–	–	–	–	6 (100%)
E. faecium ($n=1$)	0	0	1 (100%)	0	0	1 (100%)

[a]According to the NCCLS 2002 criteria.
[b]Because *E. faecalis* is intrinsically resistant to Q/D, the disk diffusion test was not performed.
[c]*E. durans*: Q/D:R, LZD:S; *E. casseliflavus*: Q/D:R, LZD:S; *E. durans*: Q/D:R, LZD:S; *E. avium*: Q/D:S, LZD: S.

Conclusion: Gram-positive infections have outnumbered gram-negative infections in clinical microbiology laboratories and resistance to first line antimicrobial agents is a major problem among gram-positive bacteria. Since all strains of enterococci are sensitive, linezolid seems to be an alternative agent for neterococcal infections regardless of the species. Both Q/D and LZD are promising agents for treatment of especially nosocomially acquired infections.

O247 Topoisomerase II mutations in susceptible pneumococci: the need for revised NCCLS levofloxacin breakpoints

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Objectives: *Streptococcus pneumoniae* strains that have reduced susceptibility to the fluoroquinolones as a result of first-step mutations in the type II topoisomerase genes, *parC* and *gyrA*, are more likely to acquire second-step mutation and become fully resistant. Detection of strains with first-step mutations is essential in order to predict the emergence of resistance and to make better treatment decisions in using FQs for treatment. Since levofloxacin (levo) is often used by laboratories to predict susceptibility to other quinolones, we evaluated the ability of current NCCLS levo susceptibility breakpoints (>4 mg/mL) to predict such isolates.
Methods: A total of 16 626 clinical isolates of *S. pneumoniae* were collected as part of a 1993–02 Canada-wide surveillance program of which 4332 had levo MICs of >1 mg/mL. The *parC* gene of 172 isolates with a levo MIC 2 mg/mL and 166 isolates with a MIC of 1 mg/mL were amplified by PCR and the nucleotide sequence determined.
Results: Of the 172 isolates with a levo MIC of 2 mg/mL, 103 (60%) had a ParC substitution (78 at Ser-79 and 25 at Asp-83). Of the 166 isolates with a levo MIC of 1 mg/mL, 31 (19%) had a substitution in ParC (27 at Ser-79 and four at Asp-83). Substitutions at Ser-79 included tyrosine, phenylalanine and alanine. Substitutions at Asp-83 included asparagines, serine, glycine and valine. Of five randomly chosen isolates with a levo MIC of 0.5 mg/mL, one had a Ser-79-Phe substitution.
Conclusion: Of 338 isolates with a levofloxacin MIC <4 mg/mL, 40% had a first-step *parC* mutation indicating that a significant number of strains harboring first-step mutations remain undetected when using the current NCCLS levo breakpoint. Although not all first step mutants would be detected, reducing levofloxacin susceptibility breakpoints to = 1 μg/mL would improve detection of strains with first-step mutations.

O248 A 4 year global evaluation of the susceptibility of *Candida* species to Fluconazole (one year for Voriconazole) by disk diffusion

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Objectives: The in vitro activity of fluconazole against 79 664 yeast isolates was determined employing a disk diffusion method recently endorsed by the NCCLS. Pathogenic yeasts were tested from mid-1997 through 2001 and study data was contributed by 89 centers in 35 countries. The in vitro activity of voriconazole was also determined against 18 569 yeasts collected in 2001.
Methods: All isolates were tested employing a commercially prepared 25 μg fluconazole disk (1 μg disk for voriconazole). Inoculum was equivalent to a 0.5 McFarland turbidity standard and the medium employed was Mueller-Hinton agar supplemented with 2% glucose and 0.5 μg/mL methylene blue. Test plates were incubated for 18–24 h, and automatically read and electronically recorded with BIOMIC Image Analysis Systems. Duplicate isolates and uncontrolled test results were not analyzed. Fluconazole zone interpretive criteria was reported as per NCCLS accepted guidelines: Susceptible (S) ≥ 19 mm, Susceptible-Dose Dependent (S-DD) 15–18 mm, Resistant (R) ≤ 14 mm.
Results: 98.4% of 52 987 *C. albicans* were susceptible to fluconazole. The highest resistance rate for *C. albicans* was found in Ecuador (8.6% of 512 isolates). There was a decrease over time in the percentage of *C. albicans* isolated, from 69.7% in 1997–98 to 63% in 2001 along with an increase in the isolation rates for C. tropicalis and *C. parapsilosis*. The overall susceptibility of fluconazole against all *Candida* species (excluding *C. krusei*) was 93.0% (S only) or 96.1% (S + S-DD). Zone diameters were reported for voriconazole but interpretive criteria have not yet been determined.
Conclusions: Disk diffusion is a rapid, reproducible, and cost-effective assay to determine the susceptibility of *Candida* species to fluconazole, and may serve as a basis for the development of disk diffusion for other antifungal agents.

Mechanisms of resistance and of antibiotic action

O249 The development of vancomycin (VAN) resistance in *Staphylococcus aureus*

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Objectives: *Staphylococcus aureus* with intermediate resistance to VAN (VISA) is rare. Strains with hetero-resistance (hVISA) have been reported to comprise up to 20% of MRSA in some parts of the world. It is possible that the hVISA phenotype represents a partially resistant stage in the development of full resistance. We have studied this by comparing the ease of selection of VAN resistance in hVISA, VAN-sensitive, methicillin-resistant (MRSA), and VAN-sensitive and methicillin-sensitive (MSSA) *S. aureus*.
Methods: Five MSSA, seven MRSA, and seven hVISA (defined by population analysis) were tested. Resistance selection was by serial daily subculture in increasing VAN concentrations in Mueller–Hinton broth for 28 days. VAN MICs were performed by NCCLS agar incorporation methodology on MH agar on isolates from day 0 (D0), 7, 14, 21, and 28 of selection. Population analyzes were performed on all strains by inoculating an overnight broth onto Brain Heart Infusion agar containing a range of vancomycin concentrations from 0 to 48 mg/L. Colonies were counted after 48 h incubation at 350°C.
Results: The table shows the number of strains of each starting phenotype acquiring vancomycin resistance after 7, 14, 21 and 28 days of VAN selection. hVISA strains developed vancomycin resistance most rapidly and all acquired resistance during the 28 days of selection. Population analysis showed that fully vancomycin susceptible strains (MSSA, MRSA) passed through a stage of hetero-vancomycin resistance during the development of resistance.

	No. of strains	Starting MIC (range)	Number of strains (%) with MIC >4 mg/L			
			Day 7	Day 14	Day 21	Day 28
MSSA	5	1–2	0 (0)	1 (20)	1 (20)	4 (80)
MRSA	7	0.5–2	0 (0)	0 (0)	3 (43)	2 (29)
HVISA	7	1–4	1 (14)	4 (57)	6 (86)	7 (100)

Conclusions: Hetero-vancomycin resistance is an intermediate stage in the development of full vancomycin resistance in *S. aureus*.

O250 Activity of quinolones against *Mycobacterium tuberculosis* DNA gyrase: inhibition of DNA supercoiling and DNA cleavage assays

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Objectives: Our objective was to study the DNA gyrase of *M. tuberculosis* for its enzymatic activity and its affinity for quinolones. Since gyrase is the only quinolone target in mycobacteria, the concentrations of quinolones necessary to inhibit the enzyme might correlated with the MICs against *M. tuberculosis*.
Methods: Because *M. tuberculosis* is a very slow growing and pathogenic bacteria, *gyrA* and *gyrB* genes of *M. tuberculosis* were cloned into pET29a and

pET19b, respectively, and expressed in *Escherichia coli* in order to produce gyrase safely in a large quantity. The concentrations necessary to inhibit the catalytic activity (IC50) of DNA gyrase of *M. tuberculosis* and to generate gyrase-mediated cleavable complex (CC50) were determined for 23 classical and new quinolones.
Results: Soluble 97 kDa GyrA and 72 kDa GyrB proteins were obtained and were free of host *E. coli* gyrase activity. Equimolar amounts of the GyrA and GyrB subunits reconstituted a functional enzyme with the activities of ATP-dependent DNA supercoiling (activity of 103 U/mg), ATP-independent DNA relaxation, and DNA cleavage. The IC50 determined for 23 quinolones correlated with the MICs ($r = 0.87$): e.g. MIC of sparfloxacin, moxifloxacin, ofloxacin, enoxacin and nalidixic acid were 0.25, 0.5, 1, 8 and 128 mg/L, respectively, and their IC50 were 2, 5, 11, 50 and 1100 mg/L, respectively. This allows to propose the inhibition of DNA supercoiling as a test for screening the new compounds that might have antituberculosis activity, a IC50 lower than 15 mg/L predicting activity against *M. tuberculosis*. The cleavable-complex assay was non valid without ATP because the DNA relaxation activity hampered the visualization of the cleaved band. The CC50 determined in presence of ATP were 4 mg/L for sparfloxacin, 6 mg/L for moxifloxacin, 20 mg/L for ofloxacin, and 6 mg/L for ciprofloxacin.
Conclusion: The affinity for quinolones of *M. tuberculosis* DNA gyrase correlated with MICs. These findings will allow to investigate quinolone structure-activity relationship and to screen compounds with promising activity against *M. tuberculosis*.

O251 Optimal timing of prophylactic antibiotics in surgery corresponds with a peak in penicillin-binding protein expression in infecting bacteria

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Objectives: The probability of surgical-wound infections in clean-contaminated surgery is the lowest when prophylactic antibiotics are given between 2 h before incision and the time of incision. Administration between incision and 3 h after incision and between 3 and 24 h after incision increases the risk for infection 2.4 and 5.8 times, respectively (NEJM, 1992, 326, 281–6). Surgical wound infections are often related to foreign bodies such as sutures, drains and prosthesis. This study aims to evaluate penicillin-binding protein expression (the target of beta-lactam antibiotics) during the first 24 h of in vivo foreign body related infection.
Methods: One hundred and sixty polyurethane fragments were contaminated with a homogenously methicillin-resistant *Staphylococcus epidermidis* and implanted subcutaneously in a rat model for foreign body infections. The samples were explanted at 8 time points (0, 15, 120, 240, 360, 720 and 1440 min) during the first day of infection and the expression of *pbp2a* gene was quantified with RT quantitative PCR as the cDNA/gDNA quotient as previously described (BBRC, 2002).
Results: After implantation, *pbp2a* expression increased and peaked between 15 and 60 min, whereafter a progressive decrease was observed ($P < 0.001$ for evolution, 1-way ANOVA; with expression at $t = 15$, 60 and 120 min being significantly higher than expression at $t = 1$ day, Bonferroni test). The mean peak expression after 60 min of in vivo infection was 11.1 times higher than the mean through expression after 24 h of in vivo infection ($P < 0.0001$, t-test).
Discussion: *pbp2a* has a higher expression during early but not during late in vivo foreign-body associated growth. Thus, the prophylactic administration of antibiotics in the hours before incision results in peak tissue-concentrations when the molecular target have their highest expression level. When the prophylactic antibiotics are given too early, tissue concentrations will already be below the MIC at the time of infection. When the prophylactic antibiotics are given too late, the molecular targets of these antibiotics will already have a lower expression level.

O252 Emergence and spread of CTX-M-15 producing *Escherichia coli* isolates resistant to fluoroquinolones and with a variable resistance to aminoglycosides and tetracycline

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Objectives: Molecular study of three multiresistant *E. coli* isolates (A: resistant (R) to gentamicin (GEN) and tetracycline (TET), susceptible (S) to amikacin (AMI); B: R to AMI and TET, S to GEN, and C: S to aminoglycosides (AMG) and TET) responsible for 12, 89 and 13 new cases of urinary tract infection (UTI) or colonization, respectively, in a 650 bed geriatric hospital over a 1 year period. Isolates A and B were detected in October 2001 whereas isolate C was detected in April 2002.
Methods: The plasmid-mediated multiresistance was studied by mating experiments, plasmid extraction, gene amplification and sequencing. The three epidemic isolates were compared with nine sporadic UTI *E. coli*, obtained over the same period, regarding Ramdom Amplified Polymorphic DNA (RAPD) profile, phylogenetic group and 10 virulence factors.
Results: Isolates A, B and C harbored only one plasmid of about 80 kb and two *bla* genes, TEM-1B and CTX-M-15. Although the AMG resistance phenotype was different for isolates A and B, these isolates harbored the same *aac(6′)-1b* gene which was not found in the isolate C susceptible to AMG. Isolates A, B and C displayed the same RAPD profile which differed from the eight different profiles displayed by the nine control isolates. Isolates A, B and C belonged to the phylogenetic group B2 whereas two control isolates belonged to this group, one to group B1, three to group D and two to group A. Neither *papC* and *papG* genes nor *sfa/foc* and *SfaS* genes were found in the three epidemic isolates whereas at least one of these genes was found in four out of the nine control isolates. Genes *aer*, *fyuA*, *Irp2* and the sequence TSPEA4 were found in isolates A, B and C whereas they were not found in all control isolates. Only one isolate (control) harbored the *hly* and *cuf1* genes. The two control isolates which displayed the same RAPD profile also displayed the same phylogenetic group (D) and virulence factors ($n = 5$).
Conclusion: Although the three epidemic isolates differed from each other concerning the AMG and TET resistance pattern, all the molecular studies performed strongly suggest that they belong to the same clone. The different AMG resistance pattern could be explained by a variable expression or the loss of the *acc (6′)-1b* gene, as this gene has often been found in integrons. As the epidemic clone does not appear more virulent than the eight randomly chosen clones to which the control isolates belong, other factors must have contributed to the fantastic spread of this clone.

O253 Increased prevalence of multiresistant Enterobacteriaceae during an *Enterobacter cloacae* outbreak: coincidence or transfer of resistance genes?

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Objectives: During an outbreak in an Intensive Care Unit (ICU) of an aminoglycoside-resistant, extended spectrum beta-lactamase (ESBL)-positive *Enterobacter cloacae*, an increased prevalence of several other Enterobacteriaceae with similar resistance patterns was observed. We investigated whether this could be ascribed to interspecies transfer of resistance genes.
Methods: Relatedness between *E. cloacae* was determined with amplified fragment length polymorphism (AFLP) analysis. Plasmids were isolated from 10 outbreak strains and 42 multidrug-resistant Enterobacteriaceae isolated from ICU patients during the outbreak. For restriction fragment length polymorphism (RFLP) analysis, plasmids were digested with EcoRI. Transfer of resistance was investigated by conjugation experiments. PCR and sequence analysis, using generic primers for the TEM and SHV beta-lactamase genes was performed.
Results: AFLP analysis identified that the *E. cloacae* outbreak was caused by a single clone. Aminoglycoside resistance and ESBL phenotype could be transferred separately via conjugation. Analysis of the transconjugants showed that these antibiotic resistance determinants were located on different plasmids. The specific RFLP patterns of both plasmids were observed in the epidemic clone as well as in several other Enterobacteriaceae, indicating interspecies plasmid transfer. PCR and sequence analysis revealed the presence of an SHV-12 ESBL gene in the epidemic *E. cloacae* and other Enterobacteriaceae. The precise extent of transfer of the SHV-12 ESBL- and aminoglycoside-resistance genes to other strains is currently investigated.
Conclusion: Our findings indicate the simultaneous occurrence of spread of resistance plasmids via interspecies transfer and a clonal outbreak of *E. cloacae* carrying the same plasmids. Beside relatedness between strains, characterization of transferable resistance genes deserves attention in hospital epidemiology.

The gastro-intestinal tract: the source and impact of nosocomial infections

O254 Incidence of *Clostridum difficile* associated diarrhea after lower limb surgery

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Objectives: *Clostridium difficile* is a Gram-positive anaerobic organism that is readily transmitted via hand contact. Due to the use of broad spectrum antibiotics, *C. difficile* may cause a variety of disease ranging from mild diarrhea to the potentially fatal pseudomembranous colitis. Elderly patients are more susceptible to infection with *C. difficile*, and therefore early diagnosis and treatment is essential to prevent serious complications arising. Our aim was to quantify the incidence of *C. difficile* associated diarrhea in elderly patients undergoing lower limb surgery, and to determine its outcome.
Methods: A retrospective analysis was performed of the case notes of 739 (460 female: 279 male) patients who underwent lower limb surgery (Total hip replacement, Total knee replacement, or internal fixation of a femoral fracture) between November 1999 and June 2001. Of this group 29 patients (25 female: 4 male) were identified to have suffered with *C. difficile* associated diarrhea within 6 weeks of surgery. The case notes of these patients were analyzed in depth to elucidate details of the diagnosis and management of the Clostridium infection, the presence of additional risk factors was also noted.
Results: On average, patients developed diarrhea 21 days after surgery. All patients were given three doses of intravenous cefuroxime during the perioperative period, and 21 patients received further antibiotic therapy during the postoperative period. In 23 cases treatment with metronidazole was instituted, in five cases vancomycin was commenced and no treatment was commenced in one case. The majority of cases resolved with treatment however, six patients died following infection with *C. difficile*.
Conclusion: In our series the incidence of *C. diffcile* associated diarrhea, following lower limb surgery, was 3.9% and a case fatality rate of 21% occurred. *C. difficile* associated diarrhea is a potentially fatal condition, particularly in an elderly population, who often have significant comorbidity. Greater awareness of the condition and a high index of suspicion are required for prompt diagnosis and treatment. As always, antibiotics should be used judiciously in this group of patients.

O255 Genotyping of toxin A-negative *Clostridium difficile* strains from outbreaks in different countries

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Objectives: *Clostridium difficile* has been recognized as a cause of nosocomial diarrhea and pseudomembranous colitis. The enteropathogenicity is associated with the production of enterotoxin A (308 kDa) and cytotoxin B (270 kDa). Clinical isolates usually produce both toxin A and B, but an increasing number of reports mention severe infections caused by toxin A-negative, toxin B-positive strains. The aim of this study was to investigate the relatedness of toxin A-negative strains from eight different countries.
Methods: Reference strains of *C. difficile* (29 known serotypes) were included in this study as control strains. Tox A-negative, tox B-positive strains ($n = 59$) were obtained from outbreaks in eight countries. Two to three tox A-negative strains of each country were used (total $n = 18$) for genotyping and for determination of MLS resistance. In addition, 10 unrelated tox A-positive, tox B-positive isolates of *C. difficile* were included. Tox A-negative strains were defined as strains with a deletion in the toxin A gene. PCR-ribotyping, based on amplification of the intergenic spacer region between the 16S and the 23S rRNA genes, was used as the standard typing technique. This technique was compared to amplified fragment length polymorphism (AFLP) using restriction, selective amplification and analysis with the GelComparII system. Clindamycin (MLS) resistance was tested by a PCR for the *ErmB* gene.
Results: Using PCR ribotyping, 29 reference strains yielded 25 genotypes. Indistinguishable were serotypes H and K, A7 and A11, and A14 and S4. AFLP discriminated 25 types of 27 different serotypes, but was not able to separate type A7 from type A11, and A14 from S4. PCR-ribotyping produced eight genotypes among the 10 unrelated strains, and five types for the 18 tox A-negative strains. AFLP yielded eight genotypes among the 10 unrelated strains, and six types for 16 tox A-negative strains. Clindamycin resistance was found in 13 of 18 tox A-negative strains, and in two of the 10 unrelated strains ($P = 0.008$).
Conclusions: Outbreaks of tox A-negative strains in different countries are not due to clonal spread of a specific *C. difficile* strain. The results also indicate that AFLP has a higher discriminatory power than PCR-ribotyping especially for tox A-negative *C. difficile* strains. In addition, a remarkable high percentage (72%) of tox A-negative *C. difficile* strains shows resistance to clindamycin due to the presence of the *ErmB* gene.

O256 Fusidic acid and metronidazole in the treatment of *Clostridium difficile*-associated diarrhea: a double-blind randomized controlled trial

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Objective: To compare the efficacy of fusidic acid and metronidazole for treatment of a first episode of *Clostridium difficile*-associated diarrhea (CDAD).
Methods: An investigator-initiated prospective, randomized, double-blind, multicentra trial was performed in 131 patients with a first episode of CDAD. The patients were randomized to receive either fusidic acid, 250 mg orally t.i.d. or metronidazole, 400 mg orally t.i.d. for 7 days. After 1 week of treatment, clinical and bacteriological cure were determined on days 8–13 and clinical and bacteriological recurrences on days 35–40.
Results: Fifty-nine evaluable patients received fusidic acid and 55 received metronidazole. On the first follow-up, 83% of the patients in the fusidic acid group and 93% of the patients in the metronidazole group were clinically cured ($P = 0.116$). There was no difference in bacteriological cure (78 vs. 77%; $p = 0.939$) at that time. On the second follow-up, clinical symptoms reoccurred in 27% of patients treated with fusidic acid and in 29% of patients treated with metronidazole ($P = 0.971$). Bacteriological recurrence was seen in 13% in the fusidic acid group and in 10% in the metronidazole group. Bacteriological persistence was seen in 9% and 16%, respectively. There was no difference in side-effects.
Conclusions: Fusidic acid was shown to be as effective as metronidazole in the cure of a first episode of CDAD both in clinical and microbiological terms.

O257 The clinical outcome of bloodstream infection due to extended spectrum beta-lactamases producing *Klebsiella pneumoniae*: a case-control study

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Background: The bloodstream infections due to extended spectrum beta-lactamases (ESBL)-producing *Klebsiella pneumoniae* are increasingly being recognized. The current study was conducted to evaluate treatment outcome of bloodstream infection due to ESBL-producing *K. pneumoniae*.
Methods: Database at the Clinical Microbiology Department was reviewed to identify the patients with *K. pneumoniae* bacteremia. The blood isolates of *K. pneumoniae*, stored from January 1998 to April 2002 in deep freezer, were tested for ESBL production by NCCLS guidelines and/or double-disk synergy test. Sixty patients with bacteremia due to ESBL-producing *K. pneumoniae* (case patients) were compared with 159 matched control patients with bacteremia due to non-ESBL-producing *K. pneumoniae*. Medical records of the case and control patients were reviewed.
Results: There were no significant differences in age, sex, APACHE II score, and the primary site of infection between case and control group. Complete clinical response rate, evaluated at 72 h after initial antibiotic treatment, was higher in control group (13.3 vs. 40.3%, P-value < 0.001), whereas treatment failure rate was higher in cases (33.3 vs. 11.9%, P-value < 0.001). Time to defervescence was longer in cases than controls (mean day $\pm$ standard deviation, 6.05 ± 3.15 vs. 3.43 ± 2.27; P-value < 0.001). Overall 7 day mortality rate was 20% (12/60) in cases and 15.7% (25/159) in controls (P-value $= 0.451$). Overall 30-day mortality rate was 30% (18/60) in cases and 24.5% (39/159) in controls (P-value $= 0.410$). When evaluating patients with bacteremia due to ESBL-producing organism, excluding patients who

received inadequate definitive antibiotic treatment, the 30 day mortality for delayed effective antibiotic treatment was not higher than that for initial effective antibiotic treatment (9.1 vs. 11.1%, *P*-value = 1.000).
Conclusions: In *K. pneumoniae* bacteremia, patients infected with ESBL producer had higher initial treatment failure rate than patients with non-ESBL producers. However, production of ESBL was not associated with higher mortality if antibiotic treatment was adjusted appropriately when antimicrobial susceptibility results were returned.

O258 *Vibrio cholera* can survive and grow in free-living *Acanthameba castillanii*

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It is widely accepted that the aquatic environment is the reservoir of toxigenic *V. cholera* and that aquatic habitats can support survival of toxigenic bacterial clones by providing protective or nutrient microabode. Due to the fact that cholera is considered as a waterborne disease it has been suggested that a symbiotic relation between different plankton and the bacterium exists. However, the reservoir of survival and multiplication of *V. cholera* between epidemics are fare from completely disclosed. *Acanthameba* species are free-living ameba commonly found in natural aquatic systems. As part of bio-films in aquatic environments, free-living ameba and bacteria are involved in complex interactions and it is known that *Acanthameba* species are environmental hosts for several human pathogens. To further add light to the ecological niche of *V. cholera*, *V. cholera* strain O139 was cocultivated with *Acanthameba castillanii* to find out if a symbiotic relation could be identified. It was found that bacteria survived and multiplied at least during 7 days in the presence of ameba. On the contrary if ameba was excluded, *V. cholera* disappeared totally from cultures after 4 days. Bacteria that grow and survived in ameba were found to be intracellularly located. The localization of bacteria was in the cytoplasmic compartment of ameba and not in vacuols as could be expected. Six days post infection of ameba, cytolysis occurs. In conclusion, these results give additional insights to the ecological niche of *V. cholera* and might contribute to the understanding of the bacterial behavior between epidemics.

Vaccine and other approaches to control tuberculosis

S260 Comparative genomics as a tool for tuberculosis vaccine design

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The availability of complete genome sequences of the leading human pathogens, *Mycobacterium tuberculosis*, *M. lepre* and *M. bovis*, the progenitor of the vaccine strain *M. bovis* BCG, provides us with an unprecedented knowledge base. Armed with powerful informatics tools and extensive databases, including those of the human and mouse genomes, we are now in a stong position to develop new vaccines to prevent tuberculosis using hypotheses derived in silico. These hypotheses can be tested experimentally using appropriate animal models to evaluate novel vaccine candidates that include, among others, various recombinant BCG strains, attenuated derivatives of *M. tuberculosis*, combinations of purified antigens, adjuvants and nucleic acid vaccines.

S261 The challenges encountered in the clinical evaluation of new vaccines, including TB immunogens, are both scientific and operational

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The challenges during early development are largely technical, and frequently candidates cannot be manufactured on the scale required because scientific obstacles prevent production of a sufficiently pure and stable lot for use in a clinical trial. An understanding of pathogenesis and the availability of relevant animal models in which to assess safety and immunogenicity are essential; identification and access to these models can present scientific and operational challenges, respectively. The appropriate population for the first safety trial will depend on the intended target population for Phase III. It is critical to have a valid measure of immunogenicity when selecting candidate immunogens/regimens to proceed to Phase III. Cellular immune responses are difficult to quantify with accuracy, and candidate TB vaccines face the same scientific challenges that HIV and malaria immunogens do in this regard. For field trials to assess efficacy, the site and population will be determined firstly by whether the aim is to prevent, or treat, disease. Knowledge of the local epidemiology is critical to planning the sample size. For the result to have validity, a high proportion (>85%) need to be retained in follow-up, and this can be a considerable operational challenge in more mobile populations. Other logistic issues include storage and distribution of supplies. Taking a candidate forward from the laboratory to evaluation in a Phase III efficacy trial requires a multidisciplinary approach from the outset with basic scientists working closely with epidemiologists and trialists, supported by regulatory and administrative experts. Many different departments within a pharmaceutical company would be involved in such an initiative, and this is mirrored in the public sector by the emergent vaccine networks. Interesting times lie ahead for TB, HIV and malaria mergent vaccine networks. Interesting times lie ahead for TB, HIV and malaria.

Tracing new faces of known bacterial resistances (Symposium arranged with the ESGARS)

S264 Spread, magnitude and actual prevalence of resistance to newer fluoroquinolones

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Broad use of fluoroquinolones, such as ciprofloxacin or norfloxacin, has been followed by the emergence of resistance, which is mainly due to mutations in the *gyrA* and *parC* genes, encoding the A subunits of the DNA gyrase and topoisomerase IV, the protein targets for quinolones. However, mutations in genes associated with a decrease in drug accumulation or an increased efflux of the drug can also contribute to the final level of quinolone resistance. Several models of microorganisms can be considered according to the effect of mutations in the above mentioned genes on the MIC of fluoroquinolones, among these we can enphasize: (i) Microorganisms such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, in which a single mutation is sufficient to cause clinically important levels of resistance to old fluoroquinolones (ciprofloxacin and norfloxacin), but not to the newer fluoroquinolones (levofloxacin and moxifloxacin); (ii) Enterobacteriaceae and *Neisseria gonorrhoeae* are among this group of microorganisms in which multiple mutations are required to generate clinically important resistance. In this group, several mutations generating clinical important level of resistance to old fluoroquinolones also affect the new fluoroquinolones to a similar extend; (iii) *Campylobacter jejuni*, in which a single mutation produces a high level of resistance to old fluoroquinolones and a moderate resistance to new fluoroquinolones; (iv) *Streptococcus pneumoniae*,in which the mutations in either the *gyrA* or in *parC* genes affect the MIC of the fluoroquinolones differently. Newer fluoroquinolones are incorporated into guidelines for treatment of

patients with lower respiratory tract infections. Fluoroquinolone resistance has recently begun to emerge in *S. pneumoniae,* albeit at low levels. In some cases this emergence has been related to the spread of a quinolone-resistant clone, but this does not seem to be the rule. Most recent reports have shown that ciprofloxacin resistance in *S. pneumoniae* from Canada, Spain and other countries during 2000 has remained at about the same levels since 1998 and that the susceptibility of this microorganism to levofloxacin, gatifloxacin and moxifloxacin is about 99%. However, in other countries such as Croatia or Hong Kong higher fluoroquinolone resistance rates have been reported. To maintain this high level of susceptibility to the newer fluoroquinolones in *S. pneumoniae* several mesures can be taken.

S265 Ketolides vs. streptococci: are bacteria changing their mind?

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In the very recent years, the map of macrolide resistance in streptococci has been updated, and more light has been shed on the different phenotypes and molecular mechanisms involved. Most data stem from investigations on the activity of telithromycin, and provide us with information about this compound, too. According to the breakpoints that have been official in Europe since July 2001, namely S (≤0.5 μg/mL), I (1–2 μg/mL) and R (>2 μg/mL), telithromycin was active against 99.4% of the strains tested in Western Europe and against 98.5% of the strains tested in Eastern and Central Europe. However, the actual MICs values are usually lower than in the erythromycin-susceptible strains, thus denoting a clear reduction in ketolide activity. The presence of an *erm(B)* gene impairs the in vitro activity of telithromycin in roughly 5% of isolates, possibly following the dimethylation of N6 amino group of adenine 2058. Mutational sequence alterations can also affect telithromycin affinity, and mutations in ribosomal proteins and in 23S rRNA – originally reported in macrolide-resistant pneumococci – are now found in macrolide-resistant *S. pyogenes*, too. Telithromycin MICs appear to be distinctly higher in the M-type erythromycin-resistant strains, and experiments with telithromycin 3H found it was pumped out by *S. pyogenes* isolates endowed with an *mef(A)* gene. Even though efflux is usually not sufficient to have *S. pyogenes* classified as 'telithromycin-resistant', the possibility of being affected by this resistance mechanism is a very disturbing finding, mostly for its possible additive effect to another mechanism of reduced susceptibility and for the possibility that this may represent a first step towards acquiring higher resistance levels (i.e. via a different resistance mechanism). Even though telithromycin does not share complete cross resistance with erythromycin and other macrolides, premarketing studies already evidenced its possibility of being affected by several resistance mechanisms, and postmarketing surveillance looks be essential to detect how this compound will behave, given that consumption of macrolides in upper respiratory tract infections is indeed widespread, and has been held responsible for the resistance upsurge in all major outbreaks so far described.

Quality control in molecular diagnostics (Symposium arranged with the ESGMD)

S268 Clinical virology and standardization in molecular diagnostics

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The introduction of molecular technologies has had an enormous impact on routine clinical virology and has totally revolutionalized the field of laboratory medicine. However, technological problems like the lack of sensitivity, false positivity and the inability to detect different genotypes of a virus with equal sensitivity, hampered the introduction of these techniques in routine diagnostics. About a decade ago, the first quality control program for blood borne viruses were initiated by the Dutch blood bank and the European study group of viral hepatitis (EUROHEP). This program has been very valuable for the introduction of amplification-based technology in routine diagnostics, but initially only focused on the blood borne viruses HBV, HCV and HIV-1. The last few years, more effort has been made to introduce these quality control programs more routinely for other viruses, like HSV, CMV, EBV and the enteroviruses. and the list of viruses is still growing. With the introduction of real-time technologies, more laboratories are currently introducing molecular diagnostics routinely, and even perform the assays on a day-to-day basis. But performing assays accurately is only one part of quality control; lack of standardization is a second problem. Only a limited number of international standards, defined for only a single genotype, have been introduced for testing blood and blood-products. and the directions into which diagnostics laboratories are moving with standardization are not very clear. Although it cannot be denied that an enormous progress has been made with the introduction of molecular diagnostics in clinical virology, some final hurdles have to be taken before standards can be used routinely (or not).

S269 The detection of *Mycobacterium tuberculosis*: does it work?

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The laboratory performance for detection of *Mycobacterium tuberculosis* complex by nucleic acid amplification tests (NAT) was evaluated by distributing proficiency panels that consisted of sputum samples which resembled smear-positive and smear-negative patient specimens, and a serial dilution of bacteria in saline. The distributions were organized by the Quality Control for Molecular Diagnostics (QCMD) TB program and the performance of different NATs was analyzed in combination with a questionnaire on the applied methods. The 2001-panel consisted of eight sputum specimens: two negative samples, two strong positive samples containing 50 000 CFU of *Mycobacterium bovis* BCG in 250 μL sputum, two samples with 6500 CFU/250 μL, and two samples with 650 CFU/250 μL. The samples with 650 CFU/250 μL were considered to represent 'smear-negative' samples. Among the four samples diluted in PBS there was one negative, one sample with 1000 CFU/mL *M. bovis* BCG, one with 100 CFU/mL, and one sample with 10 CFU/mL. The last sample was not included in the analysis. The panel was sent to 82 laboratories in 23 countries. Seventy-eight participants (95.2%) contributed a total of 85 evaluable data sets. The percentage of correct results on the eight sputum samples was 86.3%. Of the examinations of sputum specimens considered as 'smear-negatives' only 61% were reported positive. The percentage of correct results on the three PBS samples was 89%. The total number of false positive results was 11 (4.3%), these were reported in seven data sets. In 37.6% of data sets an 'in-house' NAT method was used and in 62.4% this was a commercial assay. The percentage of data sets achieving correct results on all sputum samples was 35.3 and 37.8%, respectively. For the PBS samples this was 45.8 and 41.5%. The results of this study show that the performance of detection of *M. tuberculosis* by NAT has improved since previous studies. The percentage of false-positives has decreased considerably. However, a large number of procedures still lack sufficient sensitivity for application on smear-negative samples. For reason that the amplification methods will be further introduced and accepted in the routine clinical laboratory it is essential that the quality of NATs are monitored regularly by distribution of proficiency panels by independent organizations such as QCMD.

S270 Composition, evaluation and control of external quality control panels

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Solna, S

In Clinical Chemistry the methods used often provide exact results. This is seldom the case in microbiology. From most assays relative values or only positive or negative results are obtained. Even in modern methods such as quantitative PCR, there are systematic variations between assays The absence of an absolute truth makes both the composition and quality material for external QC difficult. Different examples of such difficulties will be given in my presentation. Most important in the composition of a panel is that the material is clinically relevant. The targeted level of clinical sensitivity and

specificity when a routine clinical material is examined should be predefined. Thus, knowledge concerning normal distribution of levels of antibodies, microbes or microbial genes is needed. The actual testing of a panel is costly for the participants. Optimal information must be obtained with a minimum of samples. For some serological analyzes, the antigens used may vary so that numerical comparisons are impossible and only interpretation of clinical status may be asked for. A reference method for the component to be examined must be decided and information concerning must be given. If there are international standards available, QC material must be related to such standard materials. Further, reference laboratories outside the producer should examine any material before and after distribution to participants. Stability of the material under different, relevant conditions must also be examined. Only samples giving concordant results at the reference laboratories should normally be used. Sometimes this is not possible, and to deal with such and other problems there should always be a professional advisory board involved in the composition and evaluation of panels. Composition of forms and reports and, if used, scoring systems should also be discussed in detail in these groups. In the evaluation of results methods used by participants, result presentations and the wording used for results interpretation may vary among participants. To facilitate information and standardization of reports, the forms should as far as possible be of the multiple-choice type. Meetings in which participants are allowed to discuss panels and/or interactive web-sites are also prerequisites for creation of QC-programs that will meet the needs of the microbiological laboratories.

Pneumococcal disease: new developments

S273 Host immune response during pneumococcal carriage

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Objectives: The immune response to pneumococcal surface structures during human carriage was examined in a model of experimental human pneumococcal colonization.
Methods: Following intranasal challenge of healthy, uncolonized adults with either a 23F or 6B clinical isolate, a portion of the subjects became colonized. Serum from the colonized and uncolonized groups was used to determine the titer of antibody specific to pneumococcal surface components under consideration for a protein-based vaccine. These vaccine candidates included pneumococcal surface protein A (PspA), choline-binding protein A (CbpA), lipoteichoic acid, IgA1 protease, pneumolysin, proteinase maturation protein A, and pneumococcal surface adhesin A.
Results: Only PspA and CbpA were immunogenic in colonized subjects as determined by a statistically significant rise in the serum IgG titer measured by ELISA. The serum IgG response to PspA, shown previously to correlate with susceptibility to becoming colonized, was localized to an immunodominant region of N-terminal portion of PspA. This region is highly variable in amino acid sequence between pneumococcal strains. Despite the sequence diversity in the immunodominant regions of both PspA and CbpA, a significant strain to strain cross-reactivity in the serum IgG response following experimental human carriage was observed.
Conclusions: These findings support the need for further investigation of the human antibody response to PspA and CbpA and the potential use of these proteins as novel vaccine antigens for the prevention of pneumococcal colonization.

S274 Impact of novel and not so novel vaccines

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Pneumococcus is at all ages a major cause of morbidity and mortality worldwide. Young children have the highest rates of pneumococcal disease. In industrialized countries, the most common diseases are otitis media and sinusitis, but pneumococcus is also a common cause of invasive disease. In developing countries, pneumonia of young children is the most common disease caused by pneumococcus. Elderly and adults with chronic diseases are also at high risk for invasive pneumococcal disease. Resistance to penicillin and other antimicrobial agents is becoming common. Thus there are multiple reasons for finding efficient ways to prevent pneumococcal diseases. In 1983, licensed pneumococcal vaccine consists of 23 pneumococcal capsular polysaccharides. It is recommended to be used among elderly and individuals older than 2 years with an increased risk of pneumococcal disease. In USA, half of the elderly population has received the PS vaccine and its effectiveness against invasive pneumococcal disease has been documented. However, its effectiveness against pneumonia has been very low or zero. Furthermore, this vaccine cannot be used among young children. It does not evoke a protecting immune response at young age. The first pneumococcal conjugate vaccine was licensed in 2000 in the US and in 2001 in several industrialized countries, including the EU, for use among children 5 years of age and younger. The vaccine contains seven serotypes and it has been designed for the epidemiological situation in the US. The vaccine is highly effective against invasive disease, but it prevents also a considerable part of otitis media caused by the vaccine serotypes, and pneumonia and carriage. The coverage of the present seven-valent vaccine is not optimal for many parts of the world. Thus new conjugate vaccines containing nine or more serotypes are now in clinical phase II and phase III trials. In addition to the coverage, the price and the availability of the seven-valent vaccine have slowed down its introduction in the vaccination programs of even many rich countries, not to speak about the developing countries. The next generation pneumococcal vaccines have new antigens and novel approaches to increase the immune response. Several pneumococcal proteins that are shared by all pneumococcal strains have been tested successfully in preclinical studies and some are already in the clinical trials. It is hoped that these antigens could cover all pneumococci, and be cheaper than conjugate vaccines.

Hazards of modern life? Bacterial infection and blood borne viruses

O275 Community-acquired pneumonia management in 34 French emergency departments: a 2-week prospective survey

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Purpose: To identify management site and antimicrobials (AM) for community-acquired pneumonia patients (CAPP) managed in 34 French Emergency Departments (ED).
Methods: Consecutive CAPP with presumed community-acquired infections (CAI) were enrolled. Patients aged $\geq$18 years with CAP are analyzed for this study. The Fine severity score (FSS), as designed in the PORT study, was calculated and CAPP classified in five risk-classes but no aspect of management was discussed with doctors. Main endpoints are adequacy between (i) admission rate and FSS (ii) AM therapy and European Guidelines.
Results: In 14 days, 2856 patients with CAI, aged 52.0 ± 24.0 years, were included. In 482 (16.9%) with presumed CAP, age was 66.9 ± 21.3 years. [CI 95: 64.8–68.9; $P < 10–4$], sex ratio 60% M, 72% had $\geq$1 chronic condition and 38.2% $\geq$ 1 CAP risk factor. Their FSS is less severe than in the PORT study ($N = 54\,525$): $1 = 10.3$ vs. 9.4%; $2 = 20.4$ vs. 15.8%; $3 = 22.9$ vs. 17.9%; $4 = 34$ vs. 33.5% and $5 = 11.3$ vs. 23.2%; $P < 10–4$). Based on IDSA recommendations regarding FSS, 30.7% (classes $1 + 2$) would be ambulatory, 22.9% (3) would require a brief observation period before discharge and

45.3% (4 + 5) an hospitalization. Only 70 (14.5%), were actually discharged. Of 412 hospitalized, 148 (36%) were manageable at home ($P < 10–4$). European AM Guidelines suggest amoxicillin or macrolides in risk-free CAPP (30.7% in the present study), coamoxiclav for CAPP not severe but with a risk factor (22.9%), and 3GC ± macrolides ± fluoroquinolones in severe CAPP (45.3%). In the ED 461 antibiotics were prescribed in 375 CAPP (1.2/CAPP). Over 75% received a monotherapy (mainly coamoxiclav 48.3%, amoxicillin 12.3% and 3GC 8.0%); 22% were given an AM combination.

Conclusions: The annual incidence of CAP in the 34 EDs is estimated at 12 566 or 1.8% of non trauma patients (i) CAP admission rate in French EDs is >85% although home treatment is suggested for >50%. These differences may denote that ED doctors are unaware of the FS use and/or overcautious when CAP ambulatory treatment is considered. (ii) There are discrepancies between guidelines and actual AM therapy: coamoxiclav appears to be overprescribed, 3GC underprescribed.

Acknowledgement: We are grateful to all our colleagues for their participation and to Laboratoires Roche for their support.

O276 Community-acquired lower respiratory tract infections in 137 French emergency departments. Projections for the European Union

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Community-acquired Lower Respiratory Tract Infections (CALRTI) are frequent in emergency departments (ED) worldwide. Purposes of this prospective survey: identify the epidemiology of acute exacerbation of COPD (AECOPD) and pneumonia (CAP), and infer implications for EU.

Methods: Consecutive CARTI pts. aged ≥18 years. were included. Anthonisen criteria, Fine score and their usefulness for management were circulated among doctors. Duration of study per ED is 3 weeks or when four patients with mild/moderate CAP or AECOPD free of Chronic Respiratory Insufficiency (COPD-) are included.

Results: In 11.0 ± 5.2 days, we included 1603 CALRTI patients aged 68.2 ± 19 years, sex ratio 58% M; 844 were CAP [Fine 1–5: 18, 19, 24, 27, 10%], 554 AECOPD (215+/339–), 205 miscellaneous. AECOPD are older than CAP pts. (72.1 ± 14 vs. 66.6 ± 20.3 years; $P < 0.0005$) and more often hospital-managed (90 vs. 79.9%; $P < 0001$). However, more CAP than AECOPD receive antimicrobials (AM) in the ED (83.0 vs. 58%; $P < 0.001$); AM combination is more frequent in CAP than COPD (25.7 vs. 19.7%) and in COPD+ than COPD– (28.4 vs. 15.1%). Management site was the home, the ED-Observation Unit or the hospital in, respectively, 20.1% CAP vs. 10% AECOPD, 16.9 vs. 14% and 61.6 vs. 74.7% ($P < 0.001$). By contrast, current guidelines would recommend the home in 37% of the CAP study patients, the home after an observation period in 24% and the hospital in 37%. Based on a total of 126 588 visits in the 137 EDs, CALRTIs accounted for 2.9% of non trauma pts. with a ratio of 2 CAP/1 AECOPD. Extrapolated to the seven million (M) French ED-non trauma pts. in 2001, 98 000 CAP and 63 000 AECOPD would be expected. Assuming incidences of 12–16 CAP and 41 AECOPD/1000 population may apply to EU citizens (380 M), it would translate into 4.5–6 M CAP and ≥15 M AECOPD annually. National ED attendances in EU vary widely from ≥70% of the general population (Spain) to 23% (France) to ≥32% (UK). However, if French figures are an indication, ≥2.5% of European COPD (0375 M) and 10–20% CAP (0.45–1.2 M) may be managed in the ED.

Conclusions: (i) French EDs are the first management site for a great many CALRTI patients. This may well reflect the current situation in EU. (ii) Pending confirmation, it would be worthwhile designing LRTI management guidelines for European EDs, with emphasis on orally administered AM and home management whenever safe and appropriate.

Acknowledgement: We wish to thank all colleagues of the 137 EDs and Aventis-France for logistical support.

O277 The prevalence of Panton-Valentine leukocidin toxin in Scottish *Staphylococcus aureus*

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Objectives: There is increasing international concern about community acquired MRSA (CA-MRSA). Several CA-MRSA lineages identified outside the UK have been shown to have a particular variant of the DNA coding for methicillin resistance (SCCmec type IV) and to be able to produce the Panton Valentine leukocidin toxin (PVL). This toxin has also been reported in MSSA, and is associated with furunculosis and skin abscesses. It has also been linked to necrotizing pneumonia, which led to fatalities in children. It was our objective to screen Scottish *Staphylococcus aureus* clones for the presence of the PVL toxin.

Methods: A representative isolate of 29 MRSA and 15 MSSA Scottish PFGE defined clones were tested by PCR for the presence of the PVL gene. Subsequent testing of clonal variants was carried out if the initial screening of clonal representatives proved positive for PVL. Isolates positive for PVL were also tested for other staphylococcal toxins (exfoliative toxins A and B and Toxic Shock Toxin-1). A brief clinical history of the positive isolates was recorded.

Results: Of the 29 MRSA clones tested, three clones (Scottish MRSA (SMRSA) -116, -124 and -153) were positive for PVL. Further testing revealed that all five clonal variants of SMRSA-116, 2/11 clonal variants of SMRSA-124 and three epidemiologically unrelated SMRSA-153 isolates were positive for PVL. Of the 15 MSSA clones tested, only two epidemiologically unrelated isolates of a single clone – SMSSA-16 (a methicillin sensitive variant of EMRSA-16) were positive for PVL. None of the PVL positive isolates were positive for exfoliative toxins A or B, or Toxic Shock Toxin-1.

Conclusions: It has been shown that the PVL toxin is present in Scottish MRSA and MSSA clones, albeit in small numbers. Extrapolating our current results this would equate to 0.1% of all Scottish *S. aureus* referred to the Reference Laboratory, being positive for the PVL toxin. The clinical history indicated an association of the PVL toxin with skin infection.

O278 Diabetes and case-fatality in pneumococcal bacteremia – a 10-year population-based cohort study in Denmark

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Objectives: It has been suggested that patients with diabetes mellitus carry a higher risk of death after invasive pneumococcal disease when compared to nondiabetic patients. This is an important reason for offering pneumococcal immunization to patients with diabetes. We conducted a population-based historical cohort study to assess the impact of diabetes on case-fatality rate in community-acquired pneumococcal bacteremia (CAPBAC).

Methods: All patients with CAPBAC in North Jutland County from January 1992 to December 2001 were identified in a regional bacteraemia registry. Using the personal identification number assigned to every resident in Denmark, identification of patients with diabetes was done by cross-linkage with (i) a regional prescription database (providing information on exposure to antidiabetic drugs) and (ii) the regional hospital discharge registry. We performed a record review for all CAPBAC cases with diabetes to validate the diagnosis. Information on potential confounders was obtained from the hospital registry. Vital status at day 30 was determined through the Civil Registration System. Survival in patients with diabetes compared to patients without diabetes was assessed by logistic regression analysis.

Results: We confirmed 63 (9.1%) cases of diabetes among 689 patients admitted with CAPBAC. Ninety-four percent were type 2 diabetes and 57% were males. Patients with diabetes were significantly older (median age 71.9 years) than patients without diabetes (65.0 years). Mean blood glucose on admission was 14.3 mmol/L, and 9.5% had ketoacidosis at or during admission. The proportion with medium/high comorbidity scores was greater among diabetic patients, especially due to previous myocardiac infarction and congestive heart failure. Crude 30-day case-fatality was lower in patients with diabetes: 11.5 vs. 15.2%. After adjustment for sex, age and comorbidity, the odds ratio of 30-day case-fatality in diabetic patients was 0.53 (95% confidence interval, 0.23–1.23) as compared to the nondiabetic group.

Conclusion: Patients with diabetes and community-acquired pneumococcal bacteraemia seem to have a lower 30-day case-fatality rate compared to their nondiabetic counterparts.

O279 Bacteremic *E. coli* urinary tract infection: relationship between Quinolone susceptibility and prognosis in a series of 1429 episodes

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Background: In *E. coli*, ciprofloxacin-resistance (ciproR) has been associated with less putative uropathogenic virulence factors and invasiveness. However, little is known about the role of ciproR on the outcome of bacteremic urinary tract infections (UTI).
Objective: To evaluate the eventual association of ciproR with shock and in-hospital mortality in patients admitted with bacteremic *E. coli* UTI.
Methods: Analysis of an existing, prospectively collected database gathered during a 11-year period at Hospital Clínic of Barcelona (Spain). Several logistic regression models were built taking shock and in-hospital mortality as dependent variables. Explanatory variables included demographics, underlying diseases, prognosis of the underlying disease, neutropenia, presence of structural or functional abnormalities of the urinary tract, indwelling bladder catheter, prior antibiotic therapy, nosocomical acquisition of the infection, shock, ciproR and appropriateness of empiric therapy.
Results: A total of 1429 patients were evaluated. Mean (SD) age was 64.6 (18.3) years and 447 (31%) were male. In 249 episodes (17%), a ciproR strain was involved. 126 episodes (9%) were complicated by shock and 39 (3%) were fatal. In univariate analysis, ciproR was associated with both shock (OR = 2.43, $P < 0.0001$) and death (OR = 2.16, $P = 0.025$). Multivariate analysis selected age (OR = 1.03 per year, $P < 0.0001$), bladder catheterization (OR = 1.72, $P = 0.034$), liver cirrhosis (OR = 2.08, $P = 0.012$), neutropenia (OR = 3.69, $P = 0.007$) and ciproR (OR 1.86, $P = 0.005$) as independently associated with shock, and shock (OR 32, $P < 0.00001$), liver cirrhosis (OR = 2.76, $P = 0.045$) and a rapidly or ultimately fatal underlying disease (OR = 2.49, $P = 0.034$) as the best predictors of death. Because of shock can be an intermediate circumstance in the path to death, a second model without this variable was built, which selected age, bladder catheterization, liver cirrhosis and neutropenia but not ciproR as independent predictors of in-hospital mortality.
Conclusion: Once in the bloodstream, ciproR *E. coli* strains seem to be no less prone than their ciproS counterparts to cause severe sepsis or be associated with a fatal outcome.

O280 Bacterial pneumonia in HIV-infected patients: comparative study of *Streptococcus pneumoniae* and *Legionella pneumophila* sg. 1

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Background: Bacterial pneumonia is common in patients with HIV infection causing high morbidity and mortality. Bacterial pathogens have become more common with the use of HAART and successful prophylaxis against *Pneumocystis carinii*. Several bacterial pathogens cause pneumonia in HIV patients, *Streptococcus pneumoniae* being the most common. The incidence of *Legionella pneumonia* (LP) in HIV-infected patients is uncertain but morbidity and mortality of LP are significantly higher in HIV vs. non HIV patients.
Objective: To compare individual risk factors, clinical features and mortality of pneumonia by *S. pneumoniae* and *L. pneumophila* sg 1 in HIV-infected patients.
Methods: Data related to HIV and pneumonia were retrospectively collected. 18 HIV patients with pneumonia by *L. pneumophila* sg 1 (group 1) and 48 by *S. pneumoniae* (group 2) were included. Diagnosis was definitive in all cases.
Results: Most patients in the two groups were males with a mean age of 38.4 (group 1) and 35.4 (group 2).Among individual risk factors other than HIV infection, chemotherapy was more frequent in group 1 ($P = 0.01$). LP was more frequently nosocomial (27.8 vs. 4.2%) ($P = 0.01$). Patients in group 1 had a higher mean CD4 count ($P = 0.009$), a lower mean viral load ($P = 0.02$), had AIDS less frequently ($P = 0.04$) and received antiretroviral therapy more frequently than group 2 ($P = 0.001$). Class IV and V of Fine were more frequent in group 1 ($P = 0.004$). Dyspnea was present in 12(75%) vs. 22 (45.8%) of groups 1 and 2, respectively ($P = 0.05$). Extrarespiratory symptoms were present in 10 (62.5%) vs. 11 (22.9%) patients in groups 1 and 2, respectively ($P = 0.006$). Hyponatremia ($P = 0.001$) and an increase in CK ($P = 0.01$) were significantly more frequent in group 1. No radiological differences were seen at presentation. On outcome, clinical and radiological complications were more frequent in group 1 with respiratory failure and bilateral involvement being of note in group 1 ($P = 0.005$ and $P = 0.0009$, respectively). Hospital stay was longer in group 2 ($P = 0.03$). Lastly, mortality was significantly higher ($P = 0.01$) in those with LP.
Conclusions: Morbidity and mortality are significantly higher in HIV patients with *L. pneumonia* than *S. pneumoniae* despite a better immunological status.

O281 The impact of treatment with Pegylated interferon and ribavirin on ART prescribing in HIV-HCV coinfected patients

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Introduction: Successful treatment for HCV infection in coinfected patients may be complicated by interactions between HCV and HIV therapies. In vitro studies have shown an interaction with ddI and other NRTIs and RBV.
Methods: A prospective study of interventions in patients on stable HIV treatment who commenced HCV treatment was undertaken, monitoring interactions, side-effects and marrow support requirements, run in tandem with a pharmacokinetic substudy of RBV and NRTIs. Statistics were performed using nonparametric tests on SPSS.10.
Results: Of those patients on ART prior to commencing HCV treatment ($N = 32$), 60% were infected with HIV and HCV through IDU; 30% through blood products; 20% through sexual contact. 80% were male and mean age was 37 years (R: 24–49). All patients were virologically suppressed (VL < 50cpm) and mean CD4 count was 607 × 106/L (range 204–1219 × 106/L). All patients were on ART, min. of 3 drugs, consisting of 2NRTIs and PI/NNRTI/3rd NRTI. 45% were on d4T, 55% on ZDV, 25% on ddI, 80% on 3TC, 10% on TFV. 20% patients were interchanged from ddI to another NRTI prior to commencing HCV treatment. 30% of patients received marrow support, 85% were on ZDV; 25% of patients received erythropoetin (mean duration 4–16 weeks); 10% received blood products; and 10% received GCSF. 40% of patients who commenced HCV therapy whilst on ZDV as part of their ART regimen were interchanged to another NRTI because of hematological interactions. 10% of patients required dose modification of anti-HCV therapy (reduction in P-ifn dosage in 5% and in RBV dosage in 5%). Significant reductions in Hb or ANC were more likely if patients commenced HCV therapy on ART regimens that included ZDV ($P < 0.001$). No patients experienced lactic acidosis, pancreatitis or neuropathy. All patients have remained virologically suppressed on HCV treatment. There was a significant reduction in total CD4 count on HCV treatment (607 vs. 432 × 106/L; $P = 0.001$), however, the CD4% remained similar (32 vs. 34%; $P = 0.301$). HCV treatment response remained unaffected by the clinical interaction with similar outcomes occurring in those on all ART regimens and comparable to mono-infected patients.
Conclusion: While no patients discontinued HCV treatment because of hematological interactions with ART, it is a cause of significant morbidity in this group. Supportive interventions with erythropoetin, GCSF &/or interchange of ART where such a choice was available, were associated with a successful outcome in this cohort.

O282 Implementing prevention of mother to child HIV transmission (PMTCT) programme: challenges and lessons learned

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Introduction: Every year in Uganda, around 40 000 babies get HIV infection. The Ministry of Health started to implement a PMTCT service free of charge from January 2000. At present, five sites are operational in government hospitals.
Objective: Monitoring and evaluation of performance, intersite comparison, assessment and discussion of major problems faced.
Methodology: Counseling and testing was offered to all women attending antenatal clinic (ANC). Rapid tests were used and results were available the same day. Short antiretroviral courses (NVP) were offered in the late pregnancy period of PMTCT. Modification of obstetrical care and support on infant feeding issues were also part of the service.

Results: In the 1st year of intervention, 18 326 women were counseled, 11 995 were tested and 1531 found HIV positive. Of those positive, 891 were enrolled and started ARV and 710 have delivered in the program. Only around 50% of women – babies care came for follow up at week 6. Difference between the sites performances were evident both in absolute number and in percentage. Particularly sites which had "extra manpower" were able to counsel 92% of all new ANC attendance and to provide ARV to 32% of their estimated HIV positive population. But sites without any "extra manpower" were able to counsel only 17% of all new ANC attendance and to provide ARV to only 4% of their estimated HIV population. HIV prevalence found during PMTCT was consistent with coming from sentinel sites: Kampala sites had 13%, rural sites 10%. Use of formula milk was higher in urban setting.
Conclusion: The uptake of the PMTCT program has been fairly good. However there is need to look critically at human resource capacity of the implementing sites if the intervention has to reach of the beneficiaries.

O283 Protection against HSV-2 infection by HSV-1: a population-based transversal study

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Objectives: HSV-1 and HSV-2 share numerous antigenic determinants, raising the possibility that HSV-1, frequently acquired in childhood, may protect against subsequent HSV-2 infection. Controversial data have been reported in this respect both in transversal and longitudinal studies. The aim of the present paper is to report on HSV-1 and -2 interaction in the Swiss population, as detected in a seroprevalence study.
Methods: Three thousand one hundred and ten sera (MONICA health survey) randomly selected from the general population were assayed by type-specific ELISA (gG-based, Focus Technologies) for HSV-1 and -2 IgG. Data were examined by logistic and decision tree analysis.
Results: Overall, the prevalence was 80.5% for HSV-1 and 18.9% for HSV-2. In males, the age-adjusted odd ratio (OR) of being infected by HSV-2 when infected by HSV-1, compared to HSV-1 uninfected was 0.74 (CI95: 0.5–1.11, $P = 0.143$). In females, the corresponding OR was 0.59 (CI95: 0.40–0.86, $P = 0.006$). By multivariate analysis using all predictors, the ORs were, respectively, 0.79 (CI95: 0.52–1.23, $P = 0.30$) and 0.58 (CI95: 0.39–0.85, $P = 0.006$). By classification tree analysis, the population was split into five strata of increasing risk for HSV-2 infection using demographic predictors of HSV-2 infection. In males, only the average HSV-2 risk stratum showed a borderline reduced HSV-2 prevalence in HSV-1 positive subjects (OR: 0.62, $P = 0.032$). In females, the difference in HSV-2 prevalence in HSV-1 positive vs. negative subjects increased with the risk for HSV-2 infection in a dose dependent manner, with an OR reaching a minimum of 0.37 (CI95 0.19–0.75, $P = 0.005$) in the highest HSV-2 risk stratum.
Conclusion: In this transversal study, a reduced HSV-2 prevalence was observed in HSV-1 infected adults. This effect was mostly contributed by females at high risk for acquiring HSV-2. Our data perhaps explain controversial reports, with a protective effect demonstrated dependent on the risk for HSV-2 infection in the study population. Protection in females only is reminiscent of the results of the recent GSK gD2 vaccine.

O284 12 months Lamivudine therapy in children with chronic hepatitis B

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Introduction: The aim of the study was to assess a 12 months administration of lamivudine in children with chronic hepatitis B.
Methods: The study included 24 children (14 boys, 10 girls) aged 7–18-year-old who have not responded to interferon alfa therapy. The diagnosis was established according to current classification criteria. Lamivudine was administrated 100 mg daily for 12 months. In the course of the treatment, in four weeks time, ALT activity, hemoglobin levels, leukocyte and trombocyte counts in peripheral blood were determined. Moreover, in 12 weeks time, protrombin index and protein fraction were analyzed. Serologic markers of hepatitis B virus infection and presence of HBV- DNA were measured before, after six months and at the end of the treatment. In the whole group of the studied children a liver biopsy was performed before therapy. (histological evaluation according to Knodell and Scheuer) In the assessment of the effectiveness of the Lamivudine therapy we took into consideration the loss of HBV-DNA in serum and seroconversion in HBe/anty-HBe system as well as normalization ALT and AST activity.
Results: After 12 months applied Lamivudine inhibition of HBV replication and normalization of ALT and AST activity were observed in 10 children (41.7%); in six of them the replication was combined with seroconversion HBe/anty-HBe and normalization ALT and AST activity. 14 children (58.3%) have not eliminated HBV-DNA also have not obtained the seroconversion. However, six children presented ALT normalization, seven children decreased and one child twice increase in ALT activity. Side-effects of treatment were not observed in any of the studied children.
Conclusions: (i) One- year of Lamivudine therapy leads to inhibition of HBV replication and normalization ALT and AST in 25% children with chronic hepatitis B. (ii) Therapy is well tolerated and does not cause side-effects.

Molecular mechanisms of antibiotic resistance

O285 Mutations in 23S rRNA associated with linezolid resistance in *Streptococcus pneumoniae*

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Objectives: Linezolid, the first oxazolidinone in clinical use, is licensed in the UK for the treatment of pneumonia and thus far clinical strains of *S. pneumoniae* have all been susceptible (irrespective of their penicillin resistance phenotype). Resistance in other organisms such as enterococci and staphylococci has been due to mutations at the ribosomal target site. The object of the current study is to determine the genetic basis of linezolid resistance in laboratory-selected pneumococcal isolates.
Methods: Three pneumococcal strains were studied: SMH506, SMH155, and SMH683 with penicillin MICs of 0.004, 0.75 and 0.25 mg/L, respectively. Parental strains were subjected to daily serial subculture in Mueller-Hinton broth containing increasing linezolid concentrations for 28 days. Microbroth dilution MICs were performed by NCCLS methods for linezolid, chloramphenicol, gentamicin, tetracycline, clarithromycin, and clindamycin on isolates from days 0 and 28. The sequence of domain V of the 23S rRNA gene of the parents and mutants was obtained from PCR amplicons.
Results: Linezolid MICs of the parent strains SMH506, SMH155 and SMH683 were 0.5, 1.0 and 1.0 mg/L and 32, 32 and 4 mg/L, respectively, after 28 days of linezolid selection. SMH506 & SMH155 showed a >32 fold increase in chloramphenicol MIC with the development of linezolid resistance while SMH683 showed a >32 fold decrease in the clarithromycin & clindamycin MICs following linezolid selection. Both SMH506 and SMH155 carried the substitution G2576U in the 23S rRNA gene with an additional substitution (C2611U) in the latter strain. SMH683 carried an insertion in the 23S rRNA gene resulting in the addition of GU after C2626.
Conclusions: Selection of linezolid-resistant *S. pneumoniae* can be achieved in vitro. The development of linezolid resistance may be associated with alterations in susceptibilities to other ribosomally acting agents. Linezolid resistance in *S. pneumoniae* is associated with the mutation G2576U, which has been implicated in linezolid resistance in other organisms. Insertion of bases in the 23S rRNA gene has not previously been linked to reduced linezolid susceptibility in any organism.

O286 Over-expression of a novel RamA from *Enterobacter cloacae* confers carbapenam resistance in *E. coli*: report from the SENTRY Antimicrobial Surveillance Program

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Objective: To analyze the determinant of carbapenam resistance in an ICU clinical strain of *Enterobacter cloacae* isolated in New York City as part of the SENTRY Antimicrobial Surveillance Program (2001).
Methods: The isolate was cultured from a wound infection in a 43-year-old male in the neurosurgical ICU. A gene bank of *Enterobacter cloacae* strain

15-649I genomic DNA was constructed in the cloning vector pK18 using standard molecular biology techniques. The amplified gene bank was used to transform *E. coli* DH5-alpha and plated on media containing both ampicillin and the serine lactamase inhibitor BRL42715. All clones isolated harbored the same plasmid (pMATEcNY) which was further used to transform *Escherichia coli* SN03 (ampC negative). The insert of pMATEcNY was sequenced on both strands by the dideoxy-chain termination method with a Perkin-Elmer Biosystems 377 DNA sequencer and sequence analysis was performed using DNAstar software. MICs of various antimicrobials were determined using *E*-test strips (AB biodisk, Solna, Sweden) on Mueller-Hinton agar.
Results: Sequence analysis revealed that pMATEcNY contained an insert of 1100 bp including the entire ramA of *E. cloacae*. The sequence of ramA is unique and shows approximately 35% difference from the ramA of *Klebisella pneumoniae*. The MICs to imipenem and meropenem of *E. coli* DH5-alpha and *E. coli* SN03 containing pMATEcNY increased over 20times relative to the untransformed parent. Additionally the MIC to ceftazidime increased from 0.38 to >256 μg/ml.
Conclusions: *E. cloacae* isolate 15–649I possesses a unique RamA protein. Over-expression of the transcription factor RamA in *E. coli* causes a large decrease in it's susceptibility to carbapenems and ceftazidime.

O287 Quantitative determination of mRNA for efflux pump proteins in fluoroquinolone resistant *Pseudomonas aeruginosa* strains

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Objective: To investigate the role of efflux pumps and mutations in DNA gyrase subunits A and B and topoisomerase IV subunits C and E in fluoroquinolone (FQ) resistance in *P. aeruginosa*.
Methods: Quinolone resistance determining regions (QRDR) of *gyrA*, *gyrB*, *parC*, and *parE* genes were sequenced from 19 clinical *P. aeruginosa* strains with MICs to norfloxacin ranging from 1 to >256 mg/L). Susceptibilities to ceftazidime, amikacin, tobramycin, tetracycline and imipenem were also determined. mRNA for pump proteins for efflux pumps MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY were determined as cDNA by real-time PCR using the LightCycler.
Results: Increased amounts of mRNA for MexB was found for 10 strains, none of which had mutations in the regulatory gene mexR. mRNA for MexD was hyperproduced in five strains, one of which had a mutation in the regulatory gene nfxB. Six strains overproduced MexF, and one strain, also resistant to amikacin, hyperproduced MexY. One strain with high norfloxacin MIC (256 mg/L) did not overproduce any of the investigated pump proteins. Generally, no unequivocal correlation was seen between amount of expressed efflux pumps and resistance to known substrates for respective pump. However, all strains overproducing MexB were resistant to tetracycline. Alterations in GyrA were found in all strains, and in GyrB in four strains. Three of these strains (MICs 16, 16 and 256 mg/L, respectively) had GyrA (Thr83 to Ile) and GyrB (Thr146 to Asn). One strain, MIC of norfloxacin >256 mg/L with GyrA (Asp87 to Asn) had three alterations in GyrB (positions 234, 238 and 266). Three strains had alterations in ParE, and two of these strains had no alterations in QRDR of ParC. The third strain (MIC > 256) had ParC (Phe74 to Ile) and ParE (Glu460 to Val) alterations.
Conclusions: Hyperproduction of efflux pump proteins is common among FQ resistant strains, and many of these have no mutations in genes for the regulatory proteins. This suggests the presence of unidentified regulatory mechanisms for expression of efflux pump proteins. The results also indicate that overexpression of efflux pumps do not necessarily lead to increased resistance. Alterations in both subunits of gyrase and topoisomerse IV seem to be more important than efflux pumps in highly FQ resistant *P. aeruginosa*.

O288 Identification of a carbapenem-hydrolyzing class A beta-lactamase, KPC-2, in two clinical strains of *Enterobacter cloacae* and *Escherichia coli*

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Objectives: *Enterobacter cloacae* 7506 and *Escherichia coli* 2138 were isolated from a peritoneal liquid. Both isolates showed decreased susceptibility to imipenem and resistance to extended-spectrum cephalosporins and aztreonam, the activity of beta-lactam antibiotics being restored in the presence of clavulanic acid.
Mehods: DNA amplifications were carried out with primers designed to amplify the genes coding for the class A carbapenemases NMC-A, SME-1 and KPC-1 and for the class A penicillinases TEM-1 and SHV-1. Plasmid DNAs of *E. cloacae* 7506 and *E. coli* 2138 were prepared with a Qiagen plasmid DNA Maxi kit and were subjected to electroporation into *E. coli* Top10. Catalytic activities on imipenem and others beta-lactams were determined from crude extracts using an Uvikon 940 spectrophotometer.
Results: The MIC value of imipenem for *E. cloacae* 7506 and *E. coli* 2138 was 1 μg/mL. Accordingly, a low but significant catalytic activity was detected with imipenem from crude extracts prepared from the two strains. Sequencing of a PCR amplicon obtained by using KPC-1-specific primers revealed the presence of a blaKPC-2 gene in *E. coli* 2138 and *E. cloacae* 7506. At the amino acid level, KPC-2 was found to be a single point mutant of KPC-1 (Ser175Gly) weakly related to other class A beta-lactamases, with 54% identity to the carbepenem-hydrolyzing beta-lactamase SME-1 from Serratia marcescens S6, and 38% identity to the class A penicillinase TEM-1. The presence of a blaTEM gene along with blaKPC-2 was confirmed in *E. cloacae* 7506 and *E. coli* 2138 by specific DNA amplification and sequencing. Analysis of the plasmid preparation obtained from *E. cloacae* 7506 revealed the presence of at least two plasmids in this clinical strain. Resistance to extended-spectrum cephalosporins (cefotaxime, ceftazidime and aztreonam) but not to carbapenems (imipenem) could be transfered by electroporation of the *E. cloacae* 7506 plasmid preparation into *E. coli* Top10. The presence of blaTEM, but the absence of blaKPC-2, in the transformants was confirmed by DNA amplification. No plasmid was detectable in *E. coli* 2138, suggesting that the beta-lactamase genes blaKPC-2 and blaTEM were located on the chromosome of this strain.
Conclusion: Here, we report for the first time the presence of a carbapenem-hydrolyzing class A beta-lactamase (KPC-2) in *E. coli*, a worrying finding which underlines the possible spread of class A carbapenemases among the Enterobacteriaceae.

O289 Molecular analysis of fusidic acid resistance in *Staphylococcus aureus*

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Background: Fusidic acid is a potent antibiotic against severe gram-positive infections that interferes with the function of elongation factor G (EF-G), thereby leading to the inhibition of bacterial protein synthesis. Resistance to fusidic acid has been demonstrated to occur rapidly in vitro and has been observed with increasing frequency in vivo.
Objectives: This study was aimed at analyzing the mechanism of fusidic acid resistance in *Staphylococcus aureus*.
Methods and results: It could be demonstrated that fusidic acid resistance in *Staphylococcus aureus* results from point mutations within the chromosomal *fusA* gene encoding EF-G. Sequence analysis of *fusA* revealed mutational changes that cause amino acid substitutions in 10 fusidic acid-resistant clinical *S. aureus* strains as well as in 10 fusidic acid-resistant *S. aureus* mutants isolated under fusidic acid selective pressure in vitro. Fourteen different amino acid exchanges were identified that were restricted to 13 amino acid residues within EF-G. To confirm the importance of observed amino acid exchanges in EF-G for the generation of fusidic acid resistance in *S. aureus*, three mutant *fusA* alleles encoding EF-G derivatives with the exchanges P406L, H457Y, and L461K were constructed by site-directed mutagenesis. In each case, introduction of the mutant *fusA* alleles on plasmids into the fusidic acid-susceptible *S. aureus* strain RN4220 caused a fusidic acid-resistant phenotype. The elevated minimal inhibitory concentrations of fusidic acid determined for the recombinant bacteria were analogous to those observed for the fusidic acid-resistant clinical *S. aureus* isolates and the in vitro mutants containing the same chromosomal mutations.
Conclusions: The presented data provide evidence for the crucial importance of individual amino acid exchanges within EF-G for the generation of fusidic acid resistance in *S. aureus*.

O290 Detection of a new SHV-type ESBL in a *Klebsiella pneumoniae* clinical isolate from the Netherlands

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Objectives: A *Klebsiella pneumoniae* isolate resistant to third-generation-cephalosporins (3-GCs) was found in August 2001 in a wound culture of an ICU trauma patient admitted to a tertiary-care 1000-bed (18-bed ICU) teaching hospital situated in the Eastern part of the Netherlands. The patient had never been hospitalized before. Up to the end of 2002, *Klebsiella* strains with the same RFLP and RAPD were isolated from a total of 85 ICU patients. The index strain (*K. pneumoniae* KPN-15) was investigated as for the presence of an ESBL.
Methods: Antimicrobial susceptibility testing was performed by microdilution and interpreted according to the latest NCCLS documents. The presence of an ESBL was investigated by means of a double-disk synergy test. Isoelectric focusing (IEF), polymerase chain reaction (PCR) and sequencing were carried out by standard procedures.
Results: MICs were 128 μg/mL for both aztreonam and ceftazidime and 16 μg/mL for cefotaxime. The presence of an ESBL was also suggested by the synergies detected between clavulanic acid and 3-GCs in the disk-diffusion assay. Spectrophotometric analysis of crude sonic extracts confirmed that 3-GCs were hydrolyzed with fast kinetics. The IEF visualized a beta-lactamase with isoelectric point (pI) of 8.2. PCR was performed using primers specific for the bla(SHV) gene, and yielded a product of about 950 bp that – after sequencing – was found to code for a polypeptide differing from SHV-1 by two aminoacids, namely L31Q and E235K. The PCR product was successfully cloned in *Escherichia coli* XL10, causing an increase in the 3-GC MICs that paralleled those of *K. pneumoniae* KPN-15.
Conclusions: The L31Q mutation was already described in SHV-29, SHV-35, SHV-37 and SHV-40. The E235K mutation was described only very recently in a new enzyme (SHV-45) found in a Brazilian isolate of *K. pneumoniae*. To our best knowledge, this is the first time that both mutations are simultaneously found in the same enzyme, which must be regarded as a novel addition to the list of SHV-type beta-lactamases.

O291 A variety of CTX-M-type beta-lactamases identified among *E. coli* isolates in Greece

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Objectives: The plasmid-mediated CTX-M-type beta-lactamases constitute the most rapidly growing family of extended spectrum beta-lactamases (ESBLs). They are much more active against cefotaxime than ceftazidime and they have been identified over an extremely wide geographic area (Europe, South America, Japan and Mediterranean countries). In Greece, Mavroidi et al. have recently reported the first isolation of an *E. coli* strain producing CTX-M-3 in a Greek hospital. In our clinical laboratory, *E. coli* strains which are resistant to cefotaxime, ceftriaxone and susceptible to ceftazidime have been isolated at a rate of 7.5% in the years 2001–2002. Six non clonally related *E. coli* isolates with ESBL phenotype were analyzed for their beta-lactamase content and for the blaCTX-M carrying.
Methods: Six *E. coli* strains isolated from urine (5 strains) and pus (1 strain), were collected during the period 2001–2002 and studied. Susceptibility testing was performed by the agar diffusion (Kirby-Bauer) test and a commercial broth microdilution method (Sensititre Ltd, West Sussex, UK), using the National Committee for Clinical Laboratory Standards guidelines (M7-A5, Vol. 20, No. 2, 2000). Epidemiological typing was performed by pulse field gel electrophoresis (PFGE). Beta-lactamases were determined by isoelectric focusing (IEF) and the presence of a CTX-M gene was identified by PCR (Dutour et al. 2002, AAC, 46: 534–7). Sequencing of the blaCTX-M- PCR products were performed by MWG-THE Genomic Company.
Results: PFGE analysis showed that the six *E. coli* isolates were not clonally related. Extracts of cultures of five strains contained two beta-lactamases with pI values of 5.6 (TEM-type) and 8.6 (CTX-M-type). Three beta-lactamases with pI values of 5.6, 6.1 and 8.6 were identified from the extract of the sixth strain. Sequence analysis of the blaCTX-M PCR products identified the blaCTX-M-3 in three cases the blaCTX-M-15 in two cases and a blaCTX-M-15 derivative in one case. This enzyme differed from CTX-M-15 by one amino acid substitution in position ABL109 (Asn to Ser). This enzyme conferred a lower level of resistance to ceftazidime than the CTX-M-15.
Conclusions: It is evident that the presence of CTX-M-type ESBLs in *E. coli* isolates is established in Greece. Both blaCTX-M-3 and blaCTX-M-15 are present in our collection of strains which represents in- as well as outpatients. A probably new enzyme was also identified in one of the isolates tested.

O292 Expanded-spectrum resistance to fourth generation cephalosporins (cefpirome, cefepime) due to a four amino acid deletion in the H-10 helix of the chromosome-encoded AmpC enzyme of a *Serratia marcescens* clinical isolate

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A multiresistant *Serratia marcescens* strain was isolated from a urinary tract infection of a patient hospitalized in Nantes hospital in 2001. This isolate was resistant to amino-, carboxy- and ureidopenicillins, susceptible to cefotaxime and ceftriaxone and of intermediate susceptibility to ceftazidime and cefepime. No synergy was found between beta-lactams and clavulanic acid using the double-disk synergy test. Cloning of whole-cell DNA of this *S. marcescens* isolate gave *E. coli* clones harboring a cephalosporinase resistance phenotype. However, a high-level resistance to ceftazidime, cefepime and cefpirome was associated to susceptibility to ticarcillin and cefoxitin. Genetic analysis of the insert of one recombinant plasmid identified an Ambler class C beta-lactamase gene that encoded a 374 amino acid protein identical to the known AmpC cephalosporinase of *Serratia marcescens* S3, except for a four amino acid deletion located in the H-10 helix. Kinetic analyzes indicated that this enzyme was able to hydrolyze cefepime and cefpirome. This work underlined that resistance to the latest developed cephalosporins (cefepime, cefpirome) may occur in Enterobacteriaceae that results not only from amino acid substitutions but also from deletions in the AmpC-type beta-lactamase.

O293 Characterization of integrons carrying VIM-2 in *Pseudomonas* spp. from Portugal

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Objectives: The present study was aimed at the investigation of metallo-beta-lactamases in *Pseudomonas* isolates and characterization of associated integrons.
Methods: During the years of 2001–2002, the presence of metallo-beta-lactamases in imipenem resistant strains was investigated, using a bioassay and a PCR multiplex for blaVIM and blaIMP genes, both in clinical *Pseudomonas aeruginosa* isolates ($n = 90$), collected in different hospitals, as well as in Gram negative rods ($n = 60$) isolated from hospital wastewater. The characterization of integrons containing the metallo-enzyme genes was possible through sequencing. Four isolates of *P. aeruginosa*, previously characterized as VIM-2 producers, were also included in this study. Macrorestriction of chromosomal DNA with XbaI enzyme followed by PFGE was performed and patterns were analyzed to determine a possible interhospital spreading of the resistant strains.
Results: Four bioassay positive *Pseudomonas* spp. isolates (3 *P. aeruginosa* and 1 *P. alcaligenes*) were detected, with VIM-2 being the only identified metallo-enzyme. The association of VIM-2 to the In58 integron was found by sequencing in all the seven clinical VIM-2 producing isolates of *P. aeruginosa*. However, from these seven isolates, four different clones were detected by PFGE. A *Pseudomonas alcaligenes*, isolated from hospital wastewater, was found to produce VIM-2, with this cassette gene inserted in a class 1 integron, In56.
Conclusions: This is the first report of a VIM-2 enzyme that is inserted in an In56 integron in a nonclinical isolate and, to our knowledge, the first report of VIM-2 in *P. alcaligenes*. The finding that *P. alcaligenes*, from a wastewater sample, carried the same gene cassettes as those previously described in clinical isolates of other bacteria of human importance was an interesting observation. Although clonal spread was observed for four clinical isolates, it is of interest that several *P. aeruginosa* from different geographic regions of Portugal, during the 1995–2002 period, presented VIM-2 associated to In58 integron. Production of VIM-2 beta-lactamase presents an emerging threat of carbapenem resistance among *Pseudomonas* spp. in Portugal.

O294 Multi-drug resistance associated with MexXY expression in clinical isolates of *Pseudomonas aeruginosa* from a Texas hospital

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Objectives: Multi-drug resistant (MDR) strains of *Pseudomonas aeruginosa* (PA) are a significant therapeutic problem facing clinicians. The expression of broad substrate efflux pumps is primarily responsible for the MDR phenotypes. Four of these pumps have been characterized to date in PA. MexEF-OprN is one pump which can cause resistance to multiple classes of drugs including fluoroquinolones (FQs). In addition, overexpression of this pump correlates with a concomitant down-regulation in expression of a porin, oprD, resulting in carbapenem resistance. Recently, a hospital in Texas encountered several PA isolates resistant to both FQs and carbapenems. The purpose of this study was to determine if the mechanism responsible for this multidrug resistance involved the overexpression of MexEF-OprN.
Methods: To test this hypothesis, seven clinical isolates from the Texas hospital were analyzed and compared to a wild-type PA strain, PAO1. Antimicrobial susceptibility testing was performed by agar dilution in accordance with NCCLS guidelines. Expression of the porin, oprD, and four MDR efflux pumps (mexAB-oprM, mexCD-oprJ, mexEF-oprN, and mexXY) was measured by isolating total RNA and performing semiquantitative reverse-transcriptase PCR (RT-PCR) using specific internal primer pairs.
Results: In addition to FQ and carbapenem resistance, each isolate also displayed resistance to the aminoglycosides, gentamicin and amikacin. All seven isolates showed a decrease in the expression of oprD compared to wild-type PA PAO1. Interestingly, none of the seven clinical isolates overexpressed the mexEF-oprN pump. Instead, six isolates overexpressed the mexXY pump.
Conclusions: In conclusion, multidrug resistance to the FQs and carbapenems in these clinical isolates was not a result of the overexpression of the mexEF-oprN pump. Instead, resistance to these agents might be attributed to the overexpression of mexXY and the down-regulation of oprD expression. These data are significant because it is the first report of differential expression between the mexXY efflux pump and porin, oprD. Further studies are warranted to identify the mechanism of correlation between these two resistance mechanisms, or to determine if they are correlated at all.

New opportunities in the therapy of fungal diseases

K312 New opportunities in the therapy of fungal diseases

J. H. Rex
Macclesfield, UK

The availability of new drugs, innovative diagnostic tools, and standardized susceptibility testing methods has opened new vistas in medical mycology, but a review of these tools also highlights areas for future study. Who should be treated? This is the single largest knowledge gap. For cryptococcosis and histoplasmosis, the pace of the disease usually permits antigen-based detection methods to supplement culture-based methods reliably. However, the quicker pace of invasive candidiasis and aspergillosis means that diagnostic testing must be done serially and that test sensitivity must be high. Due to the unreliability of cultures, empirical clinical management rules have often been necessary. In a step forward, commercially supported antigen-based testing systems for aspergillosis and candidiasis have emerged and appear promising (J Antimicrob Chemother 2002; 49 : 11). These tools have not, however, gained widespread acceptance or licensure. Prophylactic therapy seems in some settings to reduce the strength of the signal detected by these nonculture-based systems, thus making the best balance between sensitivity and specificity even more elusive. What treatment is best? The laboratory view: standardized tools for testing of yeasts and moulds have facilitated interlaboratory collaborative comparisons. However, proofs of in vivo relevance of these data are few, with major knowledge gaps existing for moulds, amphotericin B, and the echinocandins (Clin Infect Dis 2002; 35 : 982). An understanding of the pharmacodynamic predictors of response is proving invaluable in reducing the complexity of this area. What treatment is best? The clinical view: new drugs have been licensed in recent years (voriconazole and caspofungin) and novel drug candidates (antibodies, transcription inhibitors, and others) are under development. But, evaluating new compounds (or combinations of compounds) remains a significant hurdle for developers, as successful comparative trials for most mycoses have usually required dozens of centers to collaborate over periods of several years. Even with such intensive efforts, only selected questions can be answered. Guidelines for clinical diagnosis have been proposed (Clin Infect Dis 2002; 34 : 7) and are definitely facilitating study implementation. But generation of data on drug activity in humans will remain slow and tedious until convincing nonculture-based diagnostic tools are available.

Antibiotics and programmed cell death in bacteria

K313 Programmed cell death in bacteria and antibiotics

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Programmed cell death (PCD), defined as an active process which results in cell suicide, refers to any form of cell death mediated by an intracellular death program, no matter what triggers it. The existence of toxin–antitoxin gene pairs (also called 'addiction modules') on extrachromosomal elements of *Escherichia coli*, and particularly the discovery of homologous modules on the bacterial chromosome, suggest that a potential of programmed cell death may be inherent in bacterial cultures. We have reported on the first described prokaryotic chromosomal PCD system. This is the *E. coli* mazEF regulatable chromosomal toxin–antitoxin module in which mazF encodes a stable toxin, MazF, and mazE encodes a labile antitoxin, MazE, that overcomes the lethal effect of MazF. In addition, we have shown that cell death mediated by the *E. coli* mazEF module can be triggered by several antibiotics (rifampicin, chloramphenicol, and spectinomycin) that are general inhibitors of transcription and/or translation. These antibiotics inhibit the continuous expression of the labile antitoxin MazE, and as a result, the stable toxin MazF causes cell death. Our recent results further increase the repertoire of antibiotics that trigger the mazEF-mediated PCD to include sulfonamides and trimethoprim, known to cause thymine starvation by inhibiting the folic acid metabolism. The effect of thymine starvation on PCD deserves a special attention. As early as 1954, Cohen and Barner discovered that a thymine auxotrophic mutant of *E. coli* undergoes cell death in response to thymine starvation. This phenomenon, called thymineless death (TLD), is also found in many other organisms from prokaryotes to eukaryotes. Though TLD has been investigated intensively, its molecular mechanism has not yet been explained. Thus, the results of our work showing that thymine starvation is a trigger for a built-in death program provide a new insight to an old enigma. In addition, the *E. coli* mazEF-mediated PCD network may have implications to this of other bacteria, including pathogenic one, and may enable to design a new generation of antibiotics targeted directly against participants in this network.

New strategies of HIV/AIDS vaccine development: from basic science to clinical trials

K314 New strategies of HIV/AIDS vaccine development: from basic science to clinical trials

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Substantial failure of HIV env-based vaccines to induce sterilizing immunity and to protect monkeys from infection with heterologus virus strains have favored the development of new strategies aimed at controlling infection and at preventing disease onset. To these goals, Tat, the transactivator of HIV-1 was chosen as a novel vaccine candidate. The Tat protein is produced very early after infection, is key to the virus life cycle and to AIDS pathogenesis, is immunogenic and well conserved among HIV subtypes in key functional and immunogenic domains. A Tat-specific immune response in humans or monkeys correlates with nonprogression to AIDS. In addition, Tat activates DC function and drives Th-1-type immune responses that are key to virus control. Vaccination of cynomolgus monkeys (Mks) with biologically active HIV-1 Tat protein or DNA has proven to be safe, immunogenic and to contain primary infection with the SHIV89.6P. In addition, protection correlates with the presence of CD8 + CTLs and CD8-mediated antiviral activity. No residual virus hidden in resting cells has been detected in any of the protected Mks either in the plasma or in lymph nodes, after two years of follow up and upon two boosts with tetanus toxoid, a stimulus known to activate virus replication. Safety and immunogenicity data have been confirmed by two new protocols and Tat (protein or DNA) has shown to be safe also in monkeys with AIDS. Based on these data, preventive and therapeutic phase I clinical trials are being started in Italy. In addition, immunological, virological and feasibility studies for phase II/III trials are being conducted in South Africa and Uganda. The results indicate that there is an high sequence homology and an extensive immune cross-recognition between Tat B clade, that is used for phase I studies in humans, and Tat from clades A, C and D viruses that are found in Uganda and South Africa, strongly supporting the concept that a Tat-based vaccine can be universally applied. Based on these data, second generation vaccines based on Tat alone or combined with structural genes and delivered by the mucosal route are being developed for preclinical testing (safety, immunogenicity, efficacy) in prime-boost regimens.

The challenge of the fungi (Symposium arranged by EFISG)

S316 What is the role of new diagnostic methods in the clinical management of invasive fungal infections

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Invasive fungal infections have emerged as an increasing cause of morbidity and mortality in immunocompromised patients. *Candida* and *Aspergillus* infections account for 80–90% of invasive fungal infections in patients with hematological malignancies, and rare fungal pathogens have been reported with increasing frequency. Early initiation of antifungal therapy is regarded to be crucial to reduce the high mortality rate of invasive aspergillosis. Cultures from blood are very rarely positive in patients with invasive aspergillosis, and even cultures from bronchoalveolar lavage fluid become positive usually at advanced stages of the disease only. Sensitive assays for the detection of *Aspergillus*- and *Candida*-specific circulating antigens have been developed and the results look promising. PCR assays have emerged as powerful tools with high sensitivity and specificity for the diagnosis of a broad variety of fungal pathogens. Major improvements have been achieved to ensure a reliable release of nucleic acids out of fungal cells. We and others could demonstrate PCR to be useful for rapid, sensitive and specific detection and identification of a variety of the most frequent and also emerging fungal pathogens. DNA extracted out of various clinical materials including whole blood, serum, plasma, tissue samples, and cerebrospinal and bronchoalveolar lavage fluid could be amplified by the use of these techniques. Recent advances including real-time PCR, which allows for the online quantification of the DNA-load, as well as the DNA-chip technology will help to establish nucleic acid-based detection methods in the routine microbiological laboratory. In a prospective study in 84 recipients of an allogeneic SCT, PCR-screening allows to identify patients at high risk for subsequent onset of invasive aspergillosis. Moreover, PCR-positivity in bronchoalveolar lavage samples taken at the time of transplant was found to be a strong predictor for invasive aspergillosis in recipients of an allogeneic stem cell transplant. Prospective studies evaluating the potential benefits of early therapy based on these methods in patients at high risk for invasive fungal infections are warranted.

S318 Emergence of antifungal drug resistance and the role of establishing standardized diagnostic assays

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During the last decades an increasing of invasive fungal infections has been detected. A substantial number of them do not respond to conventional treatments. It is obvious, that most of these infections affect to immunosupressed patients and thus, many factors can influence on the outcome of the process. One of these factors can be that the infection is caused by fungi exhibiting resistance to antifungals. In this context, the laboratory should be able to detect this situation and inform in an understandable way. Although, there are not totally accepted breakpoints for antifungals and fungi, useful information can be obtained of a standardized antifungal susceptibility test. There are several examples that can illustrate this assumption: (i) resistance of *C. albicans* and *C. glabrata* to azole drugs; (ii) intrinsic resistance of *C. krusei* to fluconazole; (iii) resistance to azole drugs of *Aspergillus fumigatus*; (iv) multiresistance of *Scedosporium prolificans*; (v) etc. These useful lessons were learned from the observation of poor outcomes and high CMIs of the respective fungi and opened the door for the development of standardized methodologies. Nowadays, these standardized methodologies allow a common language for all Mycology community. With the introduction of new classes of antifungals and the increasing detection of new fungal pathogens, susceptibility testing will be more necessary in the clinical setting and therefore the existence of standardized methodologies is essential.

Malaria

S320 Malaria during pregnancy. What do we know? Where are we now?

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Barcelona, E

The increased risk to malaria infection and disease in pregnancy is supported by consistent epidemiological observations from different endemic regions that go back to the early 30 s of the past century. The negative effects of malaria in the woman and the fetus depend of the level of prepregnancy immunity against malaria, which in general reflects the level of exposure to the infection. Thus, in areas of low and unstable endemicity malaria may be a common cause of maternal and perinatal mortality, whereas it is rarely a direct cause of death in settings of stable transmission, though it may indirectly contribute to it through the development of anemia in the woman and low birth weight in the fetus. Parity influences the susceptibility and severity of the infection with lower risk with increasing parity. In semi-immune women the placenta is a preferential site for parasite invasion which is associated with lower birth weight and prematurity of the newborn. Several host and parasite factors have been proposed to explain the increased risk of malaria during

pregnancy and specifically the placental invasion. Although, the exact mechanisms are not yet fully understood, the inflammatory response in the placental intervillous spaces associated with malaria may be instrumental in the induction of bad pregnancy outcomes through the liberation of immunological factors. The AIDS epidemic is changing the classical epidemiological observations to an increased severity of malaria disease and loss of the parity pattern of the infection. Whether malaria also worsens or accelerates HIV progression is not yet known. Malaria control during pregnancy relies on a combination of different strategies from clinical management, chemoprophylaxis through intermittent preventive treatment and insecticide treated nets. Avoiding malaria in the pregnant woman is a public health priority in endemic countries, although is rarely implemented. Further operational research and support for the implementation and execution of already available malaria control tools should be first in the agenda of international agencies.

S322 Malaria vaccines: from the laboratory to the field

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The demonstration of the (i) acquired protective immunity in adults living in endemic areas, (ii) cure of malaria patients with passive transfer of specific immunoglobulines, and (iii) protection conferred by vaccination with sporozoites attenuated by radiation, justifies the search for a malaria vaccine. Given the improbability that a vaccine directed against a single antigen will be completely protective, the preferred option is to combine several antigens of different stages of the parasite in a multicomponent multistage vaccine which is likely to protect both travelers and populations living in endemic areas. Potential technologies include recombinant proteins, synthetic peptides and DNA vaccines, the relevant genes encoding for malaria antigens being inserted into a plasmid or a live vector such as vaccinia or poxvirus. A number of human trials with several antigens and technologies have been carried out in the last 10 years. Three vaccines have undergone testing in the field in phase IIb or III trials. SPf66, including three synthetic peptides, has been extensively evaluated in different epidemiological settings; its overall efficacy was 23%, and only 2% in African infants, the most susceptible group. The circumsporozoite recombinant protein fused with the antigen S of the hepatitis B virus (RTS,S) and formulated in a potent adjuvant led to a high, but short-term, level of protection against infection and disease in Gambian adults. The first pure asexual blood-stage vaccine including three antigens of the merozoite stage (MSP1&2 and RESA, combination B) had an efficacy of 62% to reduce parasite density in Papua New Guinean children. A malaria vaccine that can reduce the burden of disease in the most affected populations is thus an achievable goal, each trial providing additional knowledge about mechanisms of protection as well as about new vaccine technology.

Evidence-based infection practice: maximizing outcome (Symposium arranged with the BIS)

S326 Evaluating performance through evidence based standards

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Although the goal of evidence based guidelines is not to create standards of care against which quality can be assessed many local and national organizations are using guidelines for this purpose. Such clinical standards, based on existing evidence, would be subsequently used as the criterion for evaluating the quality of care provided by an organization or individual units or departments. Such quality assurance may be undertaken through an internal or external peer review or through an accreditation process. In the United Kingdom, as in Australia, guidelines and clinical standards underpin much of the quality, or more recently, the clinical governance agenda. This process aims to make it a statutory responsibility for each organization to be accountable for ensuring monitoring and improving the quality of healthcare it provides. These clinical standards primarily enable identification of the essentials that need to be right in the treatment of particular conditions if outcomes for the patients are to be optimized. Standards can be set at several levels: minimal, normative and exemplary. It is important to recognize which level should be applied to any standard as minimal standards are primarily aimed at promoting basic levels of care by identifying those areas or professionals who perhaps require remedial, or in rare cases even punitive action. Outcome related standards are deemed as the 'gold standard' of performance measurement but in reality they are difficult to capture, particularly in the short term. Increasingly process or to a lesser extent structure measures are deemed more attractive, especially if they are linked through evidence to outcomes. Indeed, guidelines or care pathways will outline intervention or processes of care that lead to a desired outcome. For example in the revised British Thoracic Society Community Acquired Pneumonia Guideline (Thorax 2002) the timely administration (within 4 h of admission) of appropriate intravenous antibiotic therapy is deemed to have an important bearing for a positive outcome in patients hospitalized with severe community acquired pneumonia. This is regarded as an important, unambiguous, evidence based, simple and measurable process standard that is valued by clinicians, quality administrators and patients. Poor performance on a process measure gives a clear indication of the remedial action that is required, and this can be linked to an incentive to bring about positive change. On the other hand a commonly used crude outcome marker of death is more difficult to interpret as it is insensitive to the quality of healthcare received and can be influenced by a range of other factors. In Scotland the development of Clinical Standards has been undertaken by the Clinical Standards Board for Scotland, now renamed NHS Quality Improvement Scotland (http://www.nhshealthquality.org) and more recently by the Scottish Infection Standards and Strategy Group (SISS; http://www.rcpe.ac.uk). This work is used to illustrate standards development and implementation.

From genes to therapeutics (Symposium arranged with the ISC)

S328 Integrated computational platforms for antibiotics research: applications in target validation and compound evaluation

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The exponentially increasing number of sequenced microbes, together with the growing use of DNA microarray technologies have resulted in a flood of DNA sequences and mRNA profiling data. Here, we will present newly developed data analysis strategies that support the identification and prioritization of small molecules as development candidates for novel antibiotics. In a first step, it will be shown how large-scale genome comparisons can be used to identify and functionally characterize essential proteins that are amenable to target-based compound screening. In a second step, an in-silico reconstruction of a pathogen's genetic regulatory networks is demonstrated. This technique helps to pinpoint the promoters that are activated only for defined types of antibiotic stress, such as cell wall synthesis inhibition. Reporter assays employing cells that carry reporter genes fused to such promoters are an attractive alternative to the classical target-based assays, as they enable mechanism-specific cellular screening strategies. As opposed to the computational technologies supporting assay development, we have also developed a system that automatically determines the mechanisms-of-action of uncharacterized bioactive compounds. This technology is based on a 'reference compendium' of mRNA signature profiles triggered by well-characterized antibiotics. A data analysis technique is presented that identifies mechanism-specific marker genes. Pattern recognition algorithms enable an automated classification of the molecular mechanism employed by the compounds under investigation. The mRNA-based MOA classifications are of particular relevance for a systematic evaluation of bioactive natural products as a starting point for the development of new antimicrobial agents. It also helps in guiding

the downstream chemical development process ('MOA tracking'). In summary, it will be demonstrated that integrated computational systems continue to have a major impact on antibiotics target identification and validation, assay development and compound optimization. As such, computational biology and bioinformatics will play an important role in streamlining the antimicrobial discovery and development process.

S330 Structure in drug discovery

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The continuing investment in genomics and proteomics technologies is generating an increasing number of potential targets for therapeutic intervention. Target validation remains a serious issue, but the main challenge in drug discovery is to identify small molecules that will have the desired effect on the function of the target and then to optimize the physico-chemical and in vivo properties of the compounds to provide useful drugs. Recent advances in structural biology provide descriptions of the structure and mechanism of an increasing number of drug targets. A variety of techniques drawn from the methods of crystallography, NMR, cheminformatics, computational and medicinal chemistry allow detailed understanding of the relationship between structure and activity. When combined, these methods provide a rational approach to design that can exploit structural information to improve performance and success at both these hit identification and lead optimization phases of drug discovery. In this presentation, I will review the various methods and applications of structure based drug discovery and demonstrate that, for some targets, these methods can have a dramatic impact on the speed and effectiveness of drug discovery.

MRSA: from typing to control

O334 The HARMONY methicillin-resistant *Staphylococcus aureus* (MRSA) database

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Objectives: MRSA now comprise a global challenge to healthcare establishments. The HARMONY MRSA database was developed with European Union Grant funding to enable the tracking of strains and to inform risk assessment for control procedure design and application.
Methods: Ten countries were involved in the original project; others have joined, e.g. Ireland, Portugal, Poland, Scotland, Slovenia, Canada and Australia. Pulsed field gel electrophoresis (PFGE) has been standardized and is now published. Other techniques outlined below have been added to the database which includes details of epidemicity and virulence.
Results: Typing data on the www include phage patterns, toxin, resistotype and antibiograms so important to infection control and epidemiological studies. MecRIA typing has shown at least three introduction of the methicillin resistance gene into *S. aureus*. A novel binary typing system and FAFLP have also been used. The PFGE database is now externally accessible via the BIONUMERICS program. We are piloting the downloading of the database and ways of updating it interactively with reference centers and the ESCMID ARPAC project. MLST and SCCmec performed interactively with the Bath/Oxford Wellcome center have provided further insights into the evolution of these different MRSA. New, and further spread of MRSA EU clones are described, criteria for referral of isolates to a typing laboratory were agreed. A standardized approach to International MRSA nomenclature will permit country-specific names, the rule-sets varying according to a country's MRSA endemicity. Interactions with other EU projects such as DG SANCO's EARSS and HELICS projects are also being progressed.
Conclusions: The establishment of the MRSA database has established as esprit de cours in the EU and will inform the epidemiology, risk assessment processes and success of control measures as well as the emergence of new resistances in MRSA such as to linezolid and the glycopeptides.

O335 Evaluation of pulsed-field gel electrophoresis, random amplification of polymorhic DNA and plasmid profile analysis methods in genotyping of nosocomial MRSA isolates

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Objective: Plasmid profile analysis (PPA), random amplification of polymorphic DNA (RAPD), and pulsed field gel electrophoresis (PFGE) are three most valuable epidemiological tools for genotyping of methicillin resistant *Staphylococcus aureus* (MRSA) strains. Aim of this study was to evaluate those three methods in respect of their cost, reproducibility and discriminatory power.
Methods: Eighty one nosocomial MRSA isolates with unknown genetic relatedness from a Turkish tertiary care training military hospital were genotyped by PPA, RAPD and PFGE methods.
Results: All the isolates (100%) were typed by RAPD and by PFGE, however, eight (9.9%) isolates could not be typed by PPA since they lacked plasmid DNA. Reproducibility of all the three methods were found to be 100%. Discriminatory powers of PPA, RAPD and PFGE methods were calculated as 48.6, 61.1, 80.1%, respectively. The costs of typing per isolate were $3.9 for PPA, $7.3 for RAPD and $34.1 for PFGE.
Conclusion: Out of the three methods tested, PFGE allowed the most effective discrimination of MRSA strains. However, it was more time consuming and technically demanding, and required use of specialized and expensive equipment. Although PPA and RAPD were less discriminant than PFGE, these methods were technically simple, quick and cheaper. When PPA and RAPD were combined together they had equal discriminatory power to PFGE in this respect. So, it might be emphasized that PPA and RAPD methods could be preferred for screening purposes at first while PFGE as confirmatory test in typing of MRSA isolates.

O336 Prevention of MRSA on an ICU – analysis of the impact of body decontamination

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Objective: MRSA is endemic in most teaching hospitals in the UK. Within these hospitals, the ICU usually plays a big role in its generation, maintenance and dissemination. Conventional infection control measures have, so far, failed to control such outbreaks. In Aberdeen Royal Infirmary (ARI) an intervention based on whole body decontamination was implemented in a bid to control new cases of MRSA.
Methods: The setting was the ICU in ARI consisting of eight beds plus a five-bed overspill area. Over a period of 12 months from 1 May 2001, three protocol changes were made. Firstly, all patients admitted to the ICU were screened on admission for MRSA (nasal, throat, axilla and groin swabs). Secondly all received six hourly nasal ointment treatment (fucidin, naseptin and tetracycline rotated weekly). Thirdly, all patients had a daily bed bath in 4% chlorhexidine. Patients found to be MRSA positive were barrier nursed. Apart from admission MRSA screen and topical regimen, there was no change in intensive care or antibiotic prescribing policy throughout the period of the study. MRSA prevalence data were obtained via the laboratory computer system for the 12-month study period and the previous 24 months.
Results: In the 24 months prior to the study, there were 1232 admissions to the ICU and 194 new cases of MRSA identified in the unit. The five most common sites from which MRSA was isolated were endotracheal aspirates (902 MRSA positive), bronchoalveolar lavages (163 MRSA positive), sputum (125 MRSA positive), central line tips (86 positive) and tracheal aspirates (59 MRSA positive). During the 12-month period of the study, 691 patients were admitted and 41 new MRSA cases were identified, 22 of which were identified via the admission screen. A total of 1592 surveillance samples were submitted. Of these, 29 nasal, 27 throat, 10 groin and 6 axilla specimens were MRSA positive. The reduction in new cases from 16% (prior to intervention) to 6% (during intervention) was statistically significant ($P < 0.01$). The topical prophylactic regimen was easy to institute and well received by staff who

perceive it to have been very successful. There was no antibiogram change over the study period.
Conclusions: Instigation of MRSA screening on admission and barrier nursing of new MRSA cases combined with a topical regimen led to a significant reduction in the number of new cases of MRSA in the ICU over the 12 month study period.

O337 Sucessful infection control practices for reducing the nosocomial transmission of methicillin-resistant *Staphylococcus aureus* in a university hospital

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Objectives: Aim of the study was to evaluate the effectiveness and feasibility of a strict infection control strategy to contain cross-transmission of MRSA in a 237-bed tertiary teaching hospital in an area with a high level endemic MRSA.
Methods: Until 1998 we only occasionally detected MRSA in clinical specimens from patients admitted to our hospital. From 1999 to 2001 we gradually built a control program which in 2002 included screening of potential MRSA carriers with defined risk factors (transfer from other hospitals' ICU or nursing home, surgery in last year, previous colonization with MRSA), consistent use of alcoholic hand rub instead of washing with soap and water and decolonization of patients from whom MRSA was recovered.
Results: During 5 years period, we detected 225 patients colonized or infected with MRSA and 1100 patients with MSSA. In 1998 the prevalence rate was 4.5 patients per 1000 admissions and 8.0, 7.7, 5.7, 6.8 per 1000 admissions in 1999, 2000, 2001, 2002, respectively. Average age of patients with MRSA was 63.3, 68.7, 66.7, 69.5, 72.3 in 1998, 1999, 2000, 2001, 2002, respectively. At the beginning sputum and tracheal aspirate were the most common sample positive for MRSA, while in 2002 nasal swab became the predominant sample. Precentage of isolates classified as nosocomially acquired during stay in our hospital was 28, 44, 34, 20 and 0% in 1998, 1999, 2000, 2001 and 2002, respectively. Time from patient admission to collection of first positive sample was 9.6, 15.3, 8.1, 5.5 and 2.6 days in 1998, 1999, 2000, 2001 and 2002, respectively.
Conclusion: In a country in which MRSA has been endemic, implementation of strict infection control program (hand disinfection instead of washing, screening, isolation precautions, decolonization)is essential in preventing intrahospital MRSA transmissions.

O338 Relationship between methicillin-resistant *S. aureus* outbreaks in two Scottish hospitals

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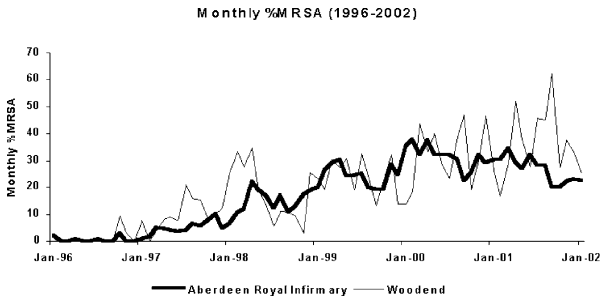
Objective: Previous work investigated the relationship between antimicrobial use and the outbreak of methicillin resistant *S. aureus* (MRSA) in Aberdeen Royal Infirmary (ARI), a large tertiary referral teaching hospital in the Grampian region of Scotland. The present study investigated the relationship between MRSA outbreaks in ARI and a long stay geriatric hospital, Woodend Hospital (WH) in the same region by means of time series analysis (TSA).
Methods: Data on monthly, nonduplicate, nonsurveillance *S. aureus* (including MRSA) and antibiotic use were collected in both hospitals from January 1996 to January 2002. Dynamic regression models were adjusted to measure relationships between data sets.
Results: Percentage MRSA in ARI rose from <1% in 1996 to 38% in February 2000, before it started to decline. WH had an independent MRSA outbreak mid-1997 to mid-1998, which then followed the ARI outbreak (Figure 1). TSA showed that: (i) Prior to 1999, percentage MRSA in ARI followed percentage MRSA in WH (lag = 1 month), whereas after 1999, percentage MRSA in ARI lead percentage MRSA in WH (lag = 1 month); (ii) percentage MRSA in ARI was closely related to previous macrolide, 3rd-generation cephalosporin and fluoroquinolone use in ARI, whereas percentage MRSA in WH was related to previous WH macrolide use only.



Conclusion: A dynamic relationship was found between MRSA outbreaks in two hospitals in the same region. The original outbreak in the long stay geriatric hospital initiated the outbreak in the teaching hospital, then the teaching hospital lead the geriatric hospital outbreak. Patient flux between hospitals can explain this relationship. Additionally, antibiotic use in each individual hospital maintained the respective outbreaks.

O339 Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* clinical isolates in Taiwan

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become an important nosocomial pathogen and the increasing prevalence is a world-wide problem. In most major hospitals in Taiwan, MRSA accounts for 50–80% of all *S. aureus* isolates. However, a nationwide molecular epidemiology of the MRSA isolates has not yet been studied extensively.
Materials and methods: During July, 2000 and October, 2001, a total of 595 MRSA clinical isolates were collected from six medical centers distributed in northern, central and southern Taiwan. The sources of specimens included blood, pus, sputum, body fluids, urine and catheter tips. The genotyping method used was pulse field gel eletrophoresis with SmaI digestion.
Results: A total of 31 genotypes with 97 type-subtypes were identified. Subtypes could be identified in seven genotypes. There were 15 genotypes in Hospitals I and VI, respectively, and six genotypes in Hospitals II, III, IV and V, respectively. 433 isolates (73%) were shown to belong to a major type (type A, 29 type-subtypes). This type was the only type prevailing in all six hospitals and the predominant clone in each hospital, accounting for 46–89% of the isolates in each hospital. Genotype C (16 type-subtypes) was the 2nd dominant type, accounting for 9% of all isolates, and was distributed in five hospitals. Genotype D (11 type-subtypes), E (five type-subtypes) and B (six type-subtypes) were distributed in five, four and three hospitals, respectively. The other 26 genotypes were minor.
Conclusion: The majority of the isolates shared a common PFGE pattern, indicating the presence of a single, epidemic MRSA clone prevailing in major hospitals in Taiwan.

O340 Absence of vancomycin-resistance in MRSA in the Tyrol, Austria 2002

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital- and community-acquired infections. It was the aim of this study to elucidate the incidence of vancomycin-intermediate sensitive (VISA) and vancomycin-resistant *S. aureus* (VRSA) in the Tyrol and to compare the MRSA rates of the various levels of medical care.
Methods: All MRSA strains isolated in 2002 in two laboratories serving all of the Tyrol (catchment area: population of 670 000) were screened for vancomycin resistance by use of a plate test: brain heart infusion agar containing 6 mg/L of vancomycin; spot inoculation with 10 µL of an McFarland 0.5 inoculum; incubation at 36°C for 24 h. Susceptible colonies were further evaluated using *E*-test. Strains that are inhibited by doses less than or equal to 4 mg/L are considered susceptible to vancomycin, those with MICs at or above 32 as resistant. For comparing MRSA rates between primary, secondary

and tertiary medical facilities, inital isolates only (one MRSA per patient) were studied using Hybase 5.1.
Results: At the 'University Lab.' a total of 6302 *S. aureus* isolates (including 1604 MRSA) and at the 'Lab. Moest' 646 isolates (including 13 MRSA) were diagnosed in 2002. All MRSA strains were susceptible to vancomycin. Methicillin resistance was found in 13.8% of all initial *S. aureus* strains cultured at the University: MRSA comprised 3.0% of *S. aureus* isolates from primary care facilities (physician offices), 13.7% of isolates from secondary care facilities (peripheral hospitals), and 19.4% of isolates from a university hospital. In this tertiary care facility 10.1% of *S. aureus* from outdoor patients were methicillin-resistant, 21.0% from patients of general wards, and 31.0% from patients of intensive care units (ICUs). MRSA comprised 1.0% of *S. aureus* isolates from primary care facilities (physician offices) sending their specimens to the 'Lab. Moest'.
Conclusions: The emergence of VISA and VRSA underscores the need for vigilance for the problem of antimicrobial resistance. Studying a closed catchment area, we were unable to demonstrate occurrence of VISA or VRSA in the Tyrol but found that the incidence of MRSA showed drastic differences between the various health care settings. Care must be taken when comparing MRSA rates between different regions without stratification for the levels of the health care settings.

O341 Are there community-acquired MRSA in Central Europe?

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Wernigerode, D

Objectives: Early detection of emergence and spread of MRSA among the community (C-MRSA).
Methods:
- MRSA surveillance by a National Reference Laboratory for Staphylococci.
- Study on nasal carriage of MRSA.
- Molecular typing of MRSA by SmaI-macrorestriction patterns, PCR detection of resistance and virulence associated genes.

Results: Among 9871 MRSA infections in Germany from 1998 to 2000, 6%, were in nonhospitalized patients attending GP's for different reasons. All of the patients affected had a previous history of hospitalization. Molecular typing attributed the majority of these MRSA to clonal groups of MRSA which are also prevalent in hospitals. Among 611 patients admitted to an ear, nose and thought ambulatory practice and to hospital emergency seven (0.9%) revealed positive for nasal carriage of MRSA. The patients had been hospitalized or at least in personal contact to hospitals during 6 months prior to this examination. Molecular typing uniformly attributed these isolates to the epidemic MRSA most frequent in nosocomial infections in this area: Barnim epidemic MRSA (syn. EMRSA 15; ST22). There have been family outbreaks of deep skin infections with *S. aureus* belonging to the same SmaI-macrorestriction cluster and possessing luk f/s (Panton-Valentine leucocidin), however, none of these isolates possessed mecA.
Conclusions: There are no indications to emergence of C-MRSA in Central Europe independent upon hospitals.

O342 Prevalence and risk factors of methicillin-resistant *Staphylococcus aureus* carriage at hospital admission

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Badalona, E

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be an important nosocomial pathogen, although the prevalence of community-acquired MRSA is rising. However, the prevalence of carriage at hospital admission and the related risk factors are not well-known.
Objective: To determine the prevalence of MRSA carriage at hospital admission and the related risk factors to define patients susceptible to screening at admission.
Methods: A transversal study was performed in a university hospital in Badalona, Spain. A randomized sample of patients admitted to hospital (excluding pediatric and obstetric patients) was screened within the first 24 h of admission (nasal swab, pressure sores and wound cultures). Demographic and clinical data, and risk factors were also collected. Preliminary results, from October 2001 to December 2002, are presented.
Results: A total of 752 patients were screened, 13 being MRSA nasal carriers (prevalence 1.7%). Significant risk factors of MRSA carriage at admission were: age ($P = 0.015$), Barthel's index ($P = 0.022$), nursing home stay within the last 12 months ($P < 0.0001$), emergency admission ($P = 0.04$)and prior hospital admission within the last year ($P = 0.008$). Other non significant variables were: diabetes, open wounds, prior antibiotic treatment within the last 6 months, hemodialysis and urinary catheter. However, only previous nursing home stay remained significant on multivariate analysis (OR: 17.7; IC 95%:2.3–136.4).
Conclusions: The prevalence of MRSA carriers at admission is low. Patients admitted from a nursing home had the highest risk of MRSA carriage at hospital admission.

O343 Epidemiological changes in methicillin-resistant *Staphylococcus aureus* infection 10 years after its initial appearance in a university hospital, Spain

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Background: Since 1990 the HUGTiP, a 600-bed university hospital, has faced an hiperendemic situation of MRSA infection.
Objectives: To analyze changes in incidence, epidemiological, clinical and microbiological characteristics of MRSA infection in HUGTiP during two time periods.
Methods: Period 1: December 1990 to December 1996; period II: July 2000 to February 2002. Epidemiological, clinical an microbiological data were collected.
Results: In period I, 462 cases of MRSA were registered and in period II, 107 new cases. The incidence of MRSA (2.9/1000 vs. 4.6/1000 admissions, $P < 0.001$) and the cases originating in the ICU (29 vs. 54.8%, $P < 0.001$) decreased significantly in the second period, while the nursing home and community-acquired cases (21.4 vs. 1.7%, $P < 0.001$)increased significantly during this period. The most frequent isolation sites of MRSA were nasal (53.7% in period I vs. 40.2% in period II), respiratory (26 vs. 30.8%), bed sores (9.9 vs. 14%), surgical wounds (9.9 vs. 15%) and blood cultures (9 vs. 18.7%). The rate of nasal carrier decreased significantly ($P = 0.01$) in the second period and the rate of bacteremia increased significantly in the second period ($P = 0.02$), although the incidence of bacteremia did not vary significantly (4 vs. 5.3/1000 admissions). In the second period a significant increase was observed in MRSA sensitive to gentamicin compared to the previous period (35.5 vs. 10.4%, $P < 0.001$). The rate of patients colonized at discharge was high and increased significantly in the second period (54.2 vs. 41.4%, $P = 0.02$).
Conclusion: Ten years after the introduction of MRSA in HUGTiP, the incidence and nosocomial-acquisition of SARM has decreased, although the nursing home and community-acquired cases has increased. On the other hand, the rate of gentamicin sensitive MRSA (35.5 vs. 10.6%, $P < 0.001$) has increased significantly. Finally, the rate of colonized discharged patients is higher, although the role this may have on community transmission of MRSA is unknown.

Tuberculosis and parasitic infections

O344 Influence of BCG vaccination on tuberculin skin test: up to what size?

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Objective: To investigate the influence of prior BCG vaccination on tuberculin skin test (TST) at different cutoff sizes, and to compare it to the influence of several indicators of a possible tuberculosis (TB) infection, in an adult population with high vaccination rate and low TB incidence.
Methods: From 1991 to 1998, all new employees at the Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, underwent a TST with 2TU RT23. A booster injection was administrated in case of an initial reaction <10 mm. Information was obtained on demographic characteristics, history of prior BCG vaccination, prior TB infection, travel to TB endemic country, employment in TB endemic country, contact with active TB and prior TSTs.
Results: Among 5117 subjects, the influence of BCG vaccination upon TST varied across categories of ages (likelihood ratio test: $P = 0.0001$): BCG vaccination was the strongest predictor of a positive TST among employees under 40 years at cutoff values of 10 mm (OR 15.0 in employees <30 years and 12.4 in employees 30–39), 15 mm (OR 9.2 and 12.2, respectively) and 18 mm (OR 4.5 and 9.7, respectively). Among employees >40, however, vaccination was a predictor only at a cutoff value of 10 mm (OR 2.8). On the other hand, BCG vaccination had no significant effect on tests greater than 20 mm whatever the age group.
Conclusion: Interpretation of tests up to 18 mm among BCG-vaccinated individuals under 40 years of age must be done with caution in areas of low TB incidence. Except for persons who have never been vaccinated, TST up to 18 mm is more likely to be the result of prior vaccination than infection and should not systematically lead to preventive treatment.

O345 Mycobacterium testing trends in clinical laboratories in Europe; a review of the UK NEQAS quality assessment scheme for Mycobacteria culture, 1992–2002

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Objectives: To evaluate the results from clinical diagnostic microbiology laboratories taking part in the UK NEQAS quality assessment scheme for Mycobacteria culture between 1992 and 2002, and to assess whether the trend of increasing use of rapid methods of mycobacterial testing is improving 'time to positive' reporting of results.
Methods: Quality assessment of Mycobacteria culture was performed. Participants are required to report on the presence of 'mycobacteria' and on the time taken to obtain a positive result. A survey questionnaire organised in 2002 addressed the use of CAMLiC (Continuous Automated Mycobacterial Liquid Culture) and molecular methods.
Results: The overall level performance with the Mycobacteria culture quality assessment specimens has remained consistently high with an average success rate of 94%. The mean 'time to positive' reporting has decreased from 24 to 17 days. A total of 269 participants in the questionnaire survey on methods returned responses by the closing date. The percentage of participants using CAMLiC methods was 70%; using DAT molecular methods on clinical specimens was 30% and using molecular methods as confirmation of identity of mycobacteria species was 35%.
Conclusions: The increase in the use of rapid culture methods for the detection of *Mycobacterium tuberculosis* has resulted in an overall reduction in 'time to positive' data as reported by participants and has provided an indication of participants' ability to meet the 21 day target for detection and identification of *M. tuberculosis* as recommended by the Centers for Disease Control and Prevention, Atlanta, GA, USA. Tuberculosis was identified as a key priority by the Chief Medical Officer of the United Kingdom in his new strategy for infectious diseases (2002). As a consequence, the results of UK participants were reviewed to provide an insight, as a baseline, into the ability of UK clinical diagnostic microbiology laboratories to meet the new challenge.

O346 Clinical evaluation of VIDAS PROBE MTB compared to MTD2 for the detection of *Mycobacterium tuberculosis*

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Objectives: To evaluate the performance of the VIDAS PROBE *Mycobacterium tuberculosis* (MTB) Test (bioMerieux, Inc., Rockland MA) as compared to the Gen-Probe (San Diego, CA) Mycobacterium Direct Test (MTD2) for the detection and identification of *Mycobacterium tuberculosis* (Mtb) complex. Additionally, a series of studies were conducted to establish specimen stability for the MTB assay. These included: fresh unprocessed specimen; decontaminated/digested (D/D) sediment; processed lysate and amplicon; fresh (refrigerated) vs. frozen (−20°C) sediment and lysate; and the impact of freeze-thaw cycles on sediment and lysate stability.
Methods: The MTB test is an automated, target-amplified test used to qualitatively detect Mtb complex rRNA from D/D sediments. The MTD2 test qualitatively detects Mtb complex rRNA from D/D sediments. Discordant results for the MTB/MTD2 comparison were resolved using conventional culture methods (inoculation to BACTEC 12B media, followed by subculture to Middlebrook 7H10 and Lowenstein-Jensen media if positive).
Results: A total of 102 sediments were tested by MTB and MTD2 with 25 positive by both methods and 75 were negative by both methods. The two discordant samples (MTB positive, MTD2 negative) were both AFB and culture positive. Therefore after resolution of discordant samples by culture, the sensitivity and specificity or the MTB test were both 100%. Results from the stability studies determined that unprocessed specimens were stable for up to 8 days at 2–8°C; D/D sediment was stable for up to 48 h at room temperature (RT) and up to 7 days at 2–8°C; processed lysate was stable up to 8 h RT and 7 days at 2–8°C; amplicon was stable 48 h at 37°C in the AMPstation instrument and 72 h stored at 2–8°C; no significant difference in the results of fresh vs. frozen sediments or lysates was observed; and five freeze-thaw cycles had no effect on the stability of sediments or lysates.
Conclusions: The VIDAS PROBE MTB Test provides rapid, amplification and detection of Mtb with a built-in internal control (no false negative results due to inhibitors). This study showed enhanced performance of the MTB assay compared to MTD2 in terms of sensitivity, automation, and technician hands on time. Specimen and procedural stability also allow increased testing and workflow flexibility.

[1]This device has not been approved by the US FDA and is not commercially available.

O347 Varying prevalence of mutations in *katG* gene codon 315 in isoniazid-resistant *Mycobacterium tuberculosis* isolates from the Middle East

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Objectives: To rapidly screen and to determine the prevalence of S315T and other mutations at codon S315 in the *katG* gene that confer clinically significant resistance to isoniazid in isoniazid-resistant *Mycobacterium tuberculosis* isolates recovered from tuberculosis (TB) patients in the Middle East.
Methods: All isoniazid-resistant ($n = 37$) and 22 -susceptible clinical *M. tuberculosis* isolates from Kuwait obtained in 2001 were analyzed. Additionally 67, 28 and 17 isoniazid-resistant and 18, seven and six -susceptible *M. tuberculosis* isolates from Kuwait, Dubai and Beirut, respectively, that were analyzed previously including the isolates with no AGC to ACC (S315T) mutation were also tested. The presence of mutations was detected by PCR amplification of the DNA region around codon S315 in the *katG* gene followed by restriction digestion with Msp I or MspA1 I to generate restriction fragment length polymorphism. The genotyping of the isolates was performed by touchdown double-repetitive-element-PCR.
Results: The mutation AGC to ACC (S315T) was detected in 22 (59%) while any mutation at codon S315 of the *katG* gene was present in 24 (65%) of 37 isoniazid-resistant isolates from Kuwait recovered from TB patients in 2001. The two isolates with other mutations at codon S315 were recovered from a patient of Middle Eastern origin and South Asian origin. The genotyping

studies showed that majority of the isolates carrying mutations at codon S315 exhibited unique DNA banding patterns. Re-analysis of isoniazid-resistant *M. tuberculosis* isolates showed that one of 21, one of 10 and two of 11 isolates (all recovered from patients of Middle Eastern origin) with no AGC to ACC mutation from Kuwait, Dubai and Beirut, respectively, contained other mutations at codon S315 of the *katG* gene. None of the susceptible strains contained any mutation at codon S315.

Conclusions: The data show that mutations other than AGC to ACC substitution at codon S315 in the *katG* gene occur more frequently in *M. tuberculosis* isolates recovered from Middle Eastern patients and should be incorporated in a rapid screen for detecting mutations in the *katG* gene conferring isoniazid-resistance.

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O348 Molecular identification of Mycobacteria using Pyrosequencing™ technology

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Objectives: We have developed a molecular method based on Pyrosequencing technology for the identification of mycobacterial species involved in atypical mycobacterial infections.

Methods: Pyrosequencing technology is a DNA sequencing-by-synthesis method based on the detection of the release of pyrophosphate (1). This technology is an easy to use technique for accurate and consistent analysis of DNA sequences. In this study we have targeted the variable *rnpB* gene encoding the RNA subunit of the enzyme RNaseP (2) in Mycobacteria for the identification of mycobacterial species involved in atypical mycobacterial infections. Atypical mycobacteria cause a wide variety of infections such as abscesses, septic arthritis and osteomyelitis and populations at risk include individuals with pre-existing lung disease and immunocompromised persons. In this study, a single PCR amplification of a region in the *rnpB* gene is followed by one sequencing reaction using Pyrosequencing. The automatically obtained sequence string was then compared with reference DNA sequences and identified.

Results: The molecular identification method was tested on DNA from reference strains as well as clinically isolates of mycobacteria. The sequencing quality of this GC-rich DNA was significantly improved by the inclusion of single-strand binding protein (SSB) added after the annealing of the sequencing primer. Sequence data and evaluation towards the *rnpB* sequence of reference strains will be presented.

Conclusion: A short region of the *rnpB* gene was shown to be highly informative and useful in rapid DNA sequencing by Pyrosequencing. Applied in clinical microbiology laboratories, this method could simplify and speed-up the identification of these slow-growing and often hard-to identify mycobacterial species.

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O349 Low sensitivity of ultrasonography for the early diagnosis of amebic liver abscess

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Objectives: Amebic liver abscess (ALA) is the most common extraintestinal manifestation of *Entameba histolytica* infection. Worldwide 100 000 people are estimated to die each year from amebic colitis and ALA. In Switzerland ALA is uncommon, usually occurring in immigrants or travelers returning from tropical and subtropical areas. Early diagnosis and prompt initiation of therapy is essential to reduce the mortality rate, which reaches 5–30% in case of complications. Abdominal ultrasonography (US) is considered to be the method of choice for diagnosing ALA, with a sensitivity of 95% reported in the literature. However, we observed several cases of ALA missed by US. The aim of this study was to investigate the clinical presentation of ALA and the sensitivity of US for the early diagnosis of ALA in a nonendemic region.

Methods: Retrospective study of all patients with liver abscess hospitalized at the University Hospital Basel between 1989 and 2002. Data were collected by chart review.

Results: Sixty-three patients were included in the study. 19 patients were diagnosed with ALA and 44 patients with pyogenic liver abscess (PLA). Clinical presentation and laboratory results were not significantly different in the two groups, except for travel and immigration history, which was more frequent in patients with ALA (84 vs. 11%, $P < 0.01$). In all patients with ALA and in 25 patients (57%) with PLA abdominal US was initially performed. Liver abscesses were identified by US in 11/19 (58%) patients with ALA compared with 23/25 (92%) of patients with PLA ($P = 0.01$). Body mass index, number, size and location of abscesses, and duration of symptoms were similar in both groups.

Conclusions: In contrast to the data reported in the literature, in our study US had a low sensitivity (58%) for the early diagnosis of ALA. This result may reflect the particular pathogenesis of ALA, which starts with ischemia and progressive necrosis, initially not detectable by US, while the abscess develops later by the confluence of microabscesses caused by the lytic effect of *E. histolytica*. Pending serologic results, the diagnosis of ALA is based on the clinical presentation, recognition of epidemiologic risk factors and early use of noninvasive imaging studies. We recommend to perform an abdominal CT-scan in patients with a history and clinical findings suggestive of ALA, if US is initially normal.

O350 A new combination with dihydroartemisinin, piperaquine, trimethoprim and primaquine, compared to atovaquone-proguanil for falciparum malaria in Vietnam

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Objectives: Dihydroartemisinin is a member of the highly active group of artemisinin antimalarials. Piperaquine is a bisquinoline, effective against chloroquine resistant parasite strains. A new antimalarial drug combination of dihydroartemisinin, piperaquine, trimethoprim and primaquine (CV8(R), DPTP) was recently launched in Vietnam for the treatment of *P. falciparum* infections. Tolerance, pharmacodynamics and efficacy of DPTP were studied and compared to atovaquone plus proguanil (Malarone(R), AP).

Methods: In a randomized open label study, patients with *P. falciparum* malaria received four dosages of DTPT (64/640/180/10 mg, $n = 82$) or three dosages of AP (1000/400 mg, $n = 79$) both over three days. Vitals and parasite counts were recorded every 8 h. After recovery and discharge, patients were followed up with weekly blood smears for 28 days. Parasite clearance kinetics were analyzed with a mixed effects population dynamic model.

Results: Tolerance to both study drugs was good, no significant side-effects were recorded. All patients recovered rapidly. The mean (95% CI) parasite elimination half life was 6.8 (6.2–7.4)h and 6.5 (6.1–6.9) h for DPTP and AP, respectively ($P = 0.41$). The mean total parasite clearance time was 35 (31–39) h and 34 (31–38) h, respectively ($P = 0.9$). Fever clearance times were 25 (22–27) h and 24 (21–26) h, respectively ($P = 0.4$). Recrudescence/reinfection occurred in 6 and 5%, respectively (OR = 0.84, 95% CI = 0.18–3.81). There were no significant differences between the two regimens with respect to tolerance, cure rate or parasite dynamics. Parasite dynamic parameters were not predictive of outcome.

Conclusions: DPTP and AP both offer equally rapid recovery and radical cure rates from falciparum malaria in an area with multidrug resistant malaria. Antimalarial combinations with dihydroartemisinin and piperaquine are cheap and effective and very promising for employment in developing countries. This is the first study which shows that AP induces equally rapid parasite clearance in vivo as artemisinin based combinations.

O351 Artesunate with mefloquine at various intervals for nonsevere falciparum malaria in Vietnam

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Objectives: Artesunate (As) is a potent drug for the treatment of *Plasmodium falciparum* malaria. Combination with mefloquine (M) is thought to delay the development of M-resistance and to prevent recrudescence which occurs after As-monotherapy. Besides, there are indications that timing of administration of M after an initial As dose is important. To study the effects of a combination of As with M in Vietnam, where M was always applied in combination

regimens, and to study the effects of different timing of M, a treatment study was performed and the mefloquine population pharmacokinetics were assessed.
Methods: Three hundred and sixty patients with uncomplicated falciparum malaria received 4 mg/kg As and thereafter were randomly allocated, in a double blind fashion, to administration of 15 mg/kg M at the same time (A), 8 h later (B) or 24 h later (C). M-placebo was used to complete three dosages for all patients. All patients were admitted until parasite and fever clearance (PCT and FCT). Thereafter they were followed for 4 weeks. Four plasma samples were drawn for measurement of M-concentrations. M-pharmacokinetics was assessed with a nonlinear mixed effect population model.
Results: All patients recovered with a mean FCT of 24 h and PCT of 42 h. Recrudescence/reinfection rates were 26, 26 and 33% for regimens A, B and C, respectively. There were no significant differences with respect to PCT, FCT and outcome. For the M-pharmacokinetics a terminal elimination half life of 319 h was entered in the population model. The mean Cmax (range) was 2888 (869–10442) mg/L, well above the generally accepted in vivo MIC of sensitive parasite strains (500 mg/L). The area under the concentration time curve (AUC) was 833771 (574327–1666844) h/mg/L. Early recrudescence was associated with a high initial parasite count ($P < 0.001$) but not with parasite clearance rate, M-Cmax or M-AUC.
Conclusions: A single dose of 4 mg/kg artesunate plus 15 mg/kg mefloquine is not effective enough in Vietnam. The parasite clearance dynamics are dominated by the effects of artesunate. A concentration effect relationship was not detected for mefloquine. The data indicate that Vietnamese parasite strains have already developed a moderate degree of mefloquine resistance despite the fact that mefloquine was always applied in combination therapy. There is no effect of different timing of the mefloquine dose within the first 24 h after an initial artesunate dose.

O352 *Mansonella perstans* infection in a cohort of HIV-infected adults in Uganda

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Objectives: Research into the effects of helminth infestation on the immune response to HIV may provide insights into the pathogenesis of HIV disease in Africa. As helminths have been shown to suppress type 1 immune responses against unrelated antigens it has been hypothesised that coinfection would have a detrimental effect on HIV disease. Filarial infections are common throughout tropical Africa and have not been studied in an HIV-infected population. The prevalence of infection with *Mansonella perstans* was studied in an established cohort of HIV-infected adults, during a longitudinal study of interactions between helminths and HIV.
Methods: Six hundred and sixty-three adults, attending one of two HIV clinics in Entebbe as part of a prospective cohort, were recruited into the current study between March 2001-March 2002. Stool and blood samples were collected, 400 mg Albendazole was administered and patients were followed up 6 months later. Examination for *M. perstans* was performed by a modification of Knott's method, on all patients at enrolment into the study. At the 6 month follow-up visit all those who had been found to be positive for *M. perstans* at enrolment were re-screened, as well as one in seven of those who were negative at enrolment. A whole blood cytokine assay was used to assess antigen-specific and mitogen-induced responses.
Results: Microfilaria of *M. perstans* were detected in 49 (7.6%) subjects. Co-infection with other helminths was common. Manual work ($P = 0.03$), lack of electricity at home ($P = 0.001$) and living further from the lake shore ($P = 0.02$) were associated with infection. On re-screening at 6 months, 68% of the positive subjects were still positive, compared with only 1% of initially negative subjects. *M. perstans* infection was strongly associated with eosinophilia ($P < 0.001$). Median CD4+ T-lymphocyte count was higher in those with *M. perstans* than in those without (369 cells/mm^3 vs. 252 cells/mm^3, $P = 0.02$). Subjects with *M. perstans* were less likely to exhibit an interferon gamma response to mycobacterial antigen in vitro ($P = 0.03$).
Conclusion: *M. perstans* infection is common among HIV-infected adults in Entebbe especially among those of lower socio-economic status. The association between *M. perstans* and a higher CD4+ T-lymphocyte count is unexpected. This observation, in conjunction with that of a diminished immune response to *Mycobacterium tuberculosis* in these patients, is the subject of further study.

O353 The therapeutic effects of eucalyptus, myrtus, ferula, artemisia, allium and urtica extracts against cutaneous leishmaniasis caused by *Leishmania* major in small white mice (out-bred)

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Protozoan parasites of the genus *Leishmania* are the causative agent of leishmaniasis. Clinical manifestations of leishmaniasis in humans range from mild cutaneous lesions to fatal visceral involvement. In this investigation, the therapeutic effects of seven herbal drugs, namely Eucalyptus, Myrtus, Ferula, Artemisia (two species), Allium and Urtica dioica on cutaneous leishmaniasis, caused by *Leishmania major*, are studied. For the purposes of this study, the *L. major* (MRHO/IR/76/ER) amastigotes that were taken from active lesions randomly were injected into the lab mice subcutaneously. It should be mentioned that promastigotes which are isolated from the N.N.N (Novy Mac Neal and Nicolle) medium could also be used. The animals were divided into three groups: (i) Test, which is treated by the use of the aforementioned herbs (ii) Control 1 which receives no treatment and (iii) Control 2 or Placebo (as the solvant of the essence or extracts was Ethanol 96, it was used to this group). After the formation of lesions which takes 1–3 months after leishmania inoculation, the specimens were prepared, the number of amastigotes were counted and the diameter of the lesions were measured. The treatment was local, twice a day for a month. At the end of the treatment period the lesions were sampled and their diameter were measured (if remained). The results showed that among the aforementioned herbs, the essence of Eucalyptus and Artemisia (one of species) reduced the diameter of lesions or caused them to disappear completely. It was also caused a remarkable reduction in the number of parasites or their complete omission. In the Placebo and Control groups no change was noticed in the size of lesions or the number of parasites. Even in most cases the number of parasites and the diameter of lesions increased. To find the effective substance existing in these essence, further investigations are recommended.

The rise and fall of macrolide antibiotics in streptococcal infections

S362 Macrolides and streptococci: an historical perspective

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Over the past few years increased rates of macrolide resistance have constantly been reported in streptococci, somehow paralleling the increased clinical use of old and news macrolides in upper respiratory tract infections. The last decade witnessed the spread of macrolide resistance in Europe, mostly in the Mediterranean countries, in both *Streptococcus pneumoniae* and *Streptococcus pyogenes*. Until very recently, susceptibility reports on *S. pyogenes* from the United States had constantly shown low levels of erythromycin resistance, but in January 2001, during a longitudinal study of schoolchildren in Pittsburgh, M-type erythromycin resistance was indeed detected in 48% of pharyngeal isolates of group A streptococci, and this clonal outbreak also affected the wider community. The role of low-level resistance and the clinical importance of the different macrolides must be carefully considered. The semisynthetic macrolides derivatives basically show the same cross resistance as erythromycin. In spite of apparently favourable in-vitro results, the use of 16-membered ring macrolides against inducible MLSB-resistant isolates should also be regarded with caution, on the basis of previous experience with the other cases of 'dissociated' resistance. It is worth reminding that the susceptibility of IR staphylococcal strains to noninducing MLSB antibiotics in the clinical setting proved to be of limited value because of the rapidity with which constitutively resistant mutants were selected from these strains, resulting in

clinical and bacteriological relapse. Owing to the totally different mechanism of resistance, the use of 16-membered ring macrolides would seem safer against those isolates endowed with an M phenotype, but this use has not been implemented by clinical evidences. Moreover, the possibility of curing low-level-resistant streptococcal strains with higher doses of macrolides should be viewed with extreme caution, and it would appear to be hazardous at the very least to apply the same considerations regarding *S. pneumoniae* and penicillin G to another species, albeit in the same bacterial genus, and to a completely different class of antibiotic.

S363 Interactions of macrolides with the streptococcal cell

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Macrolides inhibit protein synthesis by binding to the large 50S ribosomal subunit. Interactions of macrolides with the ribosome have been precisely determined in Deinococcus radiodurans and are supposed to be similar in the case of streptococcal ribosomes. Streptococci use two ways to resist macrolides: target site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target and efflux of the antibiotic. Modification of the ribosomal target confers cross resistance to macrolides and related antibiotics which have overlapping binding sites, such as the lincosamides, whereas efflux affects only some macrolides and confers a lower level of resistance. Target modification is mostly related to the synthesis of ribosomal methylases encoded by *erm(B)* or *erm(TR)* (subset of *erm(A)* class) genes. The *erm(B)* genes are usually borne by conjugative transposons and the spread of macrolide resistance in pneumococci might result both from dissemination of clonal strains, of horizontal transfer of the conjugative element and possibly from DNA exchange between strains by transformation followed by recombination. Efflux is due to the synthesis of pumps belonging to the MFS family and encoded by large transposable elements. Recently, laboratory strains and clinical isolates of streptococci, including *S. pneumoniae*, beta-hemolytic and oral streptococci harboring ribosomal mutations have been reported. In survey studies, these strains represent generally less than 2% of erythromycin-resistant streptococci but can reach higher percentages in certain countries. Various mutations of ribosomal structures, including 23S ribosomal RNA, L44 and L22 proteins have been characterized. The emergence of resistance by mutational alteration is intriguing. This type of resistance may have remained undetected in the past by lack of adequate technique or, alternatively, the resistant mutants may have emerged and spread recently. It is conceivable that the use of new long-acting macrolides with different pharmacokinetics may have contributed to modulation of the selective pressure exerted against streptococci and to selection of new resistance genotypes.

Tropical medicine in returning travelers

S366 Tropical medicine in returning travelers: dengue

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Since the early 1950s, dengue is recognized not only to cause a benign febrile illness but also to present itself as a potentially life-threatening disease. The severest forms, known as dengue hemorrhagic fever and dengue shock syndrome, are generally thought to be a result of the occurrence of two main pathophysiological changes. One is increased vascular permeability that gives rise to loss of plasma from the vascular compartment, with impending shock when plasma loss gets critical. The second change is a disorder in the hemostatic system involving vascular changes, thrombocytopenia and thrombocytopathia. Increasing evidence also suggest coagulation and fibrinolytic abnormalities to play a more pivotal role in the hemostatic changes than currently perceived. Mechanisms that may be decisive in the development of dengue hemorrhagic fever are the infecting strain or an immune enhancement in which heterotypic antibodies enhance virus replication in macrophages. Over the last 50 years both the incidence and distribution of dengue has increased steadily making it the most important of arthropod-borne viral diseases. Particularly countries in the South-east Asian region fell victim to a dramatic increase of the incidence and clinical severity of dengue with annual numbers of dengue hemorrhagic fever exceeding 200 000 in the 1990s. An increase of epidemics was also observed in several countries in the Americas following the same pattern in Asia. Infection with dengue therefore appears to be a realistic threat to travelers to endemic areas, where they have the potential to acquire and from where they could spread the virus. However, structured data on the epidemiology and clinical course of dengue infection in travelers is scarce. Several studies reported serological evidence of recent dengue virus infections in febrile patients after they returned from endemic areas to range from 7% to 45%. Studies assessing seroconversion to dengue antibodies in travelers to endemic areas demonstrated it to be substantial, although the majority of study subjects did not report to have experienced dengue-like illnesses. Nevertheless, an increased frequency of travel in conjunction with subclinical dengue infections might be of importance especially if the immune enhancement theory holds true. Continuous surveillance of imported dengue is therefore necessary to determine whether increased travel is accompanied with an increase in numbers of severe disease.

S367 Water- and food-related infections from exotic places

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Travelers may encounter manifold infections in tropical and subtropical areas. Many viral, bacterial and parasitic diseases are water-borne causes of health problems during and after stays in various countries. Disorders related to scarce water and poor hygiene, including water-washed skin infections such as lice, scabies, bacterial and fungal infections may affect low-budget travelers and long-time expatriates in rural areas. Water-based infections caused by parasites which need intermediate hosts for their development (e.g. schistosomiasis) are found among residents of endemic areas. Water-related insect and other vectors transmit diseases such as malaria, filariasis, and yellow fever which may affect persons returning or immigrating from endemic countries. An overview of the frequency and the importance of such infections among travelers, long-term expatriates and migrants provides insight into the differential diagnosis that is of growing importance to the medical practitioners in industrialized countries.

S368 Melioidosis

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Melioidosis, an infectious disease caused by the Gram negative bacterium *Burkholderia pseudomallei*, is endemic in South-east Asia and Northern Australia. The disease occurs mostly in people with underlying disorders. Clinical manifestations of melioidosis are protean, vary from benign soft tissue infection to fulminant bacteremia. Melioidosis causes diagnostic problems in endemic and especially in nonendemic areas. Laboratory diagnosis relies mainly on the isolation of *B. pseudomallei* from clinical specimens. Serological tests for early detection of melioidosis is of limited value. Many efforts have been made to develop new molecular and immunological techniques for diagnostic use. Treatment of melioidosis remains unsatisfactory. Clinical and microbiological responses are slow despite appropriate antimicrobial therapy. Two to 4 weeks of ceftazidime remains the treatment of choice for severe

melioidosis. Imipenem and sulperazone/salbactam therapy are probably as good as ceftazidime, but high dose amoxicillin- clavulanate is slightly inferior in term of clinical responses, but is equally effective at reducing mortality. Trials are on going to assess a combination of ceftazidime with cotrimoxazole (trimetroprim- sulfamethoxazole). For oral treatment, the 'conventional' regimen of chloramphenicol, doxycycline and cotrimoxazole is still the treatment of choice. Amoxicillin-clavulanate is an alternative and should be used in children and pregnant women. Failure rate of either ofloxacin or ciprofloxacin with or without azithromycin treatment or doxycycline treatment alone is high. Total treatment duration in most case should.

S369 Exotic fungus infections in the returning traveler

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Endemic mycoses are a health threat for travelers to areas endemic for these infections. In Europe histoplasmosis is the most frequent imported disease and coccidioidomycosis is an emerging imported mycose. Penicilliosis became rare since the introduction of active antiretroviral therapy in patients with AIDS. Blastomycosis, sporotrichosis cases are rare. Paracoccidioidomycosis, chromomycosis, mycetoma or entomophthomycosis are diseases of immigrants not of travelers. Histoplasmosis in Europe: An epidemiological study conducted in Europe by ECMM (coordinator: Ruth Ashbee) collected 127 cases in 12 countries (1995–99). The areas of contamination were Black Africa and Central and South America. 27% of patients had a recent infection. The most common symptoms were non specific: fever 61%, cough 38%. AIDS (41%) and exposure to birds, bats or cave (28%) were the most common risk factor. Blood culture in immunocompromised patients and histology of the lung were the most often positive methods of diagnosis. Itraconazole and amphotericin B were the usual treatment. A retrospective study on *Histoplasma duboisii* infections in France (1968–94) collected 26 cases including two in AIDS. Areas of contamination were Central and West Africa where mean duration of residence was 12 years (2–24) Fever was often absent. Localizations were: lymph nodes (13), skin (9), lungs (6), bones (5), intestine, adrenal gland, tonsil, spleen, pericardium, breast: 1 each. Disease was disseminated in 10/23 non AIDS imported cases. Diagnosis relied on biopsy, pus aspiration or sputum/BAL examination Outcome was characterized by a high relapse rate, surgery plaid a major role in recovery. Seven patients with coccidioidomycosis were diagnosed in France, mostly in recent years: three pneumopathies: two primary infections and one laboratory contamination, one skin ulceration and three meningitis. Two additional recent cases occurred in persons attending the world championship of model airplane flying in Kern County, California. Physicians should be aware of the name of the diseases, there epidemiology, areas of endemicity and symptoms. Among the causes of respiratory, skin/mucosae or CNS symptoms, 'exotic' fungal infections should be listed. The cornerstone of diagnosis is the systematic enquiry into traveling in recent (primary infection) or past history (late reactivation).

Probiotics: coming into clinical use?

S370 Clinical efficacy of probiotics: current knowledge and future challenges

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Introduction: A probiotic has been defined by the ILSI Europe working group as 'a viable microbial food supplement which beneficially influences the health of the host'. The safety and efficacy of probiotics have to be scientifically demonstrated in human studies for each strain and product.
Efficacy of probiotics: Specific probiotics have been demonstrated to be effective both in the treatment and reducing the risk of infant diarrhea in a meta-analysis. Efficacy has been shown in antibiotic diarrhea in infants. Other forms of diarrhea, such as antibiotic associated diarrhea in adults and travelers' diarrhea show promise, but further studies are needed to verify the efficacy of any of the studied strains. Significant improvement of atopic eczema in infants given diets supplemented *Bifidobacterium lactis* Bb12 or *Lactobacillus rhamnosus* GG have been reported. *L. rhamnosus* GG administered pre- and postnatally for 6 months to children succeeded to reduce the prevalence of atopic eczema to half as compared with infants receiving placebo. These results suggest that probiotics may have potential in controlling inflammatory responses, also beyond the gut. Japanese studies on *L. casei* Shirota indicate a reduction in the risk of bladder cancer and a reduction in the recurrence of superficial bladder cancer after surgery may be associated with oral *L. casei* intake. These studies need to be confirmed also in populations consuming western type diets. Other areas of intensive research with probiotics include inflammatory gut diseases. Positive results for some probiotic strains have been reported and combinations of probiotic strains have been used. However, more human studies need to be conducted prior to any conclusions on efficacy can be made.
Conclusion: Several human studies are available concerning the use and efficacy of probiotics. The evidence indicate rotavirus diarrhea and antibiotic associated diarrhea in infants as the major areas of proved efficacy. Other indications of probiotic use still need reconfirmation. All probiotic strains are different and their identification and characteristics should be well-defined. It is important to clearly identify each strain for efficacy assessment.

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S371 Probiotics: a border between efficacy and safety

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Since the days of Metchnikoff, it has been proposed that consumption of probiotics would improve the health and well-being of the host. Some known beneficial effects of probiotics include: (1) reduction in the severity and duration of microbial diarrhea; (2) prevention of traveler's diarrhea; (3) reduction in the risk of relapsis after *Clostridium difficile*-associated diarrhea; (4) prevention (or reduction) of antibiotic-associated diarrhea. Some data are now beginning to arise in regard to the usefulness of probiotics in extra-intestinal diseases (reduction of pneumonia in children with cystic fibrosis; stimulation of antibody reaction in vaccination, etc.). Still now beneficial effects of some probiotics are based on intuitive hypothesis. The results of studies, often carried out without randomized protocols, have been rather conflicting, and, so far, no consistent picture has emerged. Clinical diseases due to deleterious metabolic effects have never been reported. However, in order to take full advantage of the beneficial effects of probiotics, we ought to know more about efficacy and safety. The border between these parameters is still unclear.

S373 Future directions for probiotics in clinical medicine

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Probiotic agents are nonpathogenic, live microorganisms, found among the normal flora, that produce a positive effect on the health and well being of the host. Studies have shown potential medical benefits using probiotics for treatment and prevention of a variety of infections on mucosal surfaces, including pediatric gastroenteritis and vaginitis. The future of probiotics will depend in part on further elucidation of basic mechanisms, allowing scientists and clinicians to maximize their health benefits. Some evidence points to a potential role for probiotic agents in the prevention of sexually transmitted diseases and HIV-1 transmission. Recent studies suggest that the production of H_2O_2, rather than the particular species of *Lactobacillus*, is the essential factor for regulation of the vaginal flora. Hydrogen peroxide-generating *Lactobacillus acidophilus* at high concentrations may also be viricidal for

HIV-1. In the future, lactobacilli may play an important role in protecting women against several sexually transmitted diseases. Probiotics may also aid in the control of multiresistant organisms on mucosal surfaces. Anecdotal reports demonstrate that probiotic strains can be used to displace multiresistant organisms, such as methicillin-resistant *Staphylococcus aureus* on decubitus ulcers. The alarming increase of inappropriate antibiotic use and bacterial resistance, along with renewed interest in ecological methods to prevent infections, will make will make this an attractive field for future research. The stimulatory effect of probiotics on mucosal and systemic immunity and their effect as immunomodulators suggest that probiotics may also aid in vaccine development. This is supported by studies demonstrating adjuvant activity and enhanced immune responses from orally administrated *Lactobacillus* GG. More experimental and clinical studies are needed to clarify the role of probiotics as immunomodulators, not only for gastrointestinal infectious diseases but also for inflammatory and allergic conditions. With further research, this traditional medical therapy may still prove to be one of our most effective tools against new and emerging pathogens that continue to defy modern medicine in the twenty-first century.

Short course on antibiotic treatment

S374 Short course antibiotic treatment: the rationale

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Short course antibiotic (AB) treatments may have potential positive economical and ecological impacts. Three facts have already been established (i) increasing AB usage in many countries, with an important percentage of undue usage (ii) increasing bacterial resistance (R), and (iii) clear causality relationship between AB usage and R. AB policies should include decreasing undue usage, control of transmission of bacteria carrying R factors and optimal usage for established indications. Too long AB courses must be considered as undue usage, contributing to extension of R. Shortening AB courses may help (i) decrease AB pressure in the environment (ii) improve compliance and (iii) control extension of R. Achieving short courses is feasible in many common situations as far as the optimal drug is chosen on the following bases: activity against strains carrying R factors, pharmacokinetics, selective potential, and cost-effectiveness. Above all, the most important parameter to be taken into consideration is the choice of a dose and dosing regimen in adequacy with the pharmacokinetic/dynamic properties of the drug, with the primary goals of avoiding underdosing and allowing a true cidal effect. This is a primary requirement to make short courses feasible. Indeed, it has been demonstrated that underdosing is a significant risk factor for selection of AB R strains. The efficacy of shortened courses has been demonstrated in many common situations. However, more clinical information is needed to generalize that concept to the most frequent indications for AB therapy. New AB compounds should demonstrate their ability to reduce the length of therapy. A positive impact of short courses of AB treatment on bacterial resistance has been established through an epidemiological study (Guillemot, JAMA, 1998), clinical trials (Shrag, JAMA, 2001; Harbarth, Circulation, 2000) and an intervention study (Guillemot, ICAAC, 2001). Therefore, shortened courses of AB treatment together with the use of optimal doses should be considered as a key component of any national policy for judicious use of AB, keeping in mind the need to preserve the quality of care. Finally, attention should be paid in the future to demonstrate the cost/effectiveness of strategies aiming at reducing duration of AB therapy, taking into account not only the economical short-term impact, but also, the long-term beneficial effect on bacterial R.

Hospital water – is it safe? (Symposium arranged with the HIS)

S379 Invasive aspergillosis and hospital water

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With the continuing increase in the number of severely immunocompromised patients, hospitals are faced with the growing problem of invasive aspergillosis and other opportunistic fungal infections. Since treatment of these infections is difficult and the outcome is often fatal, preventive measures are of major importance in the control of invasive filamentous fungal infections. Nosocomial invasive aspergillosis is thought to be primarily airborne, however, despite the use of appropriate hospital air filtration systems, the incidence of this infection continues to increase. Together with the lack of genetic relatedness between airborne strains and those causing invasive disease, it is tempting to speculate about the existence of other routes of transmission. There has been an increase in data supporting hospital water as a potential source of filamentous fungi and, in particular, *A. fumigatus*. Furthermore, earlier suggestions that a wet route of transmission might exist can be found in anecdotal reports linking invasive aspergillosis with aspiration of contaminated surface water in near-drowning patients. Molecular characterization of *A. fumigatus* isolates recovered from water (at various locations inside and outside the hospital) and patients has been performed to establish if such a wet route of transmission exists. *A. fumigatus* strains genotyped by amplified fragment length polymorphism showed genotypic relatedness between clinical isolates recovered from two patients and strains isolated from water. These results suggest that patients with invasive aspergillosis can be infected by strains originating from water. How the transmission of waterborne *A. fumigatus* takes place, is not known. Aerolization may play a role in this transmission taking into account the site (lungs) of infection. The recent findings might have significant impact on the measures that are taken to prevent invasive aspergillosis in high-risk patients. Additional measures directed to water quality and activities leading to aerolization of water might be required. Restriction of exposure of high-risk patients to contaminated aerosolized water, such as showers, has been suggested. It should be emphasized that the level of contamination of hospital water varies between hospitals and countries, depending on the source of the water. Further characterization of the relative importance of each route of transmission is required to allow implementation and evaluation of preventive measures.

HIV treatment: state of the art 2003

S384 Complications of antiretroviral drugs

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Identification and characterization of drug toxicity, i.e. clinical presentation, spectrum of severity, and risk factors, has become a priority as prolonged utilization of antiretroviral drugs makes long-term side-effects a critical issue for the management of HIV-infected patients. Data from the Swiss HIV cohort study demonstrated that more than two-third of patients presented one or more clinical or laboratory abnormalities, which could have been caused by antiretroviral drugs. In the last few years, metabolic disorders (hyperlipidemia and insulin resistance) and abnormalities in body fat distribution (lipodystrophy) have increasingly drawn the physician's attention. In a experimental model, protease inhibitors were shown to inhibit proteosomal degradation of nascent apolipoprotein B. This finding supports the role of protease inhibitors in the development of hypertriglyceridemia and high cholesterol level. The increasing age of the HIV-infected population and the high prevalence of

hyperlipidemia raise a concern about a possible increase of cardiovascular morbidity. The recent observation of increased incidence of angina pectoris and myocardial infarction in patients from the HOPS cohort requires confirmation from the analysis of larger databases. Lipodystrophy, which was initially attributed to protease inhibitors, has also been observed in patients treated with a double combination of nucleoside reverse transcriptase inhibitors. We and others have shown a strong association between lipodystrophy and the use of stavudine. Potential new toxicities, such as bone disorders, underscore the complexity of the issue, and the possibility that long-term exposure to antiretroviral agents may unmask additional adverse effects. Drug toxicity may cause discontinuation of treatment or failure. Patients should be carefully monitored for side-effects of antiretroviral treatment to prevent loss of adherence and the development of viral resistance.

S385 Therapeutic drug monitoring

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The use of Therapeutic Drug Monitoring (TDM) has received an increasing amount of interest during the last years. Some national guidelines (e.g. France, United Kingdom, the Netherlands) have now included TDM as part of the diagnostic set up for a patient, but development of TDM in other countries is still in its infancy. Recently, consensus has been reached among HIV pharmacologists about the current knowledge how to perform TDM of antiretroviral agents. TDM may be particularly useful in the following situations:

- Virological failure
- Intoxication
- Drug – drug interactions
- Nonadherence

There are numerous studies showing that drug levels are related to antiviral effect, but this has not yet resulted in a consensus on a therapeutic range for these agents. Nevertheless, data have now been presented of two randomized controlled clinical trials showing that TDM of nelfinavir and indinavir in treatment-naïve patients improves the therapeutic outcome of these patients over 1 year. In contrast, disappointing results have been presented of TDM in second-line regimens, but these studies had significant methodological flaws to draw any conclusions. It is generally expected, however, that use of TDM in second-line or salvage regimens (i.e. when drug resistance may be present) will only be useful if also information is known regarding resistance (the IQ concept). Several studies are now underway that will give us information how to use TDM in the near future.

Pharmacodynamics of antibiotics

O386 Comparative pharmacodynamics of ABT492 and levofloxacin with *Staphylococcus aureus* in an in vitro dynamic model

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Objective: To compare the kinetics of killing/regrowth of *S. aureus* exposed to ABT492 and levofloxacin (LFX) over wide ranges of the ratio of area under the curve (AUC) to MIC.
Methods: A clinical isolate of methicillin-resistant *S. aureus* (MICs 0.02 of ABT492 and 0.6 mg/L of LFX) was exposed to bi-exponentially decreasing concentrations of ABT492 (a distribution half-life of 2.1 h, an elimination half-life of 23 h) and monoexponentially decreasing concentrations of LFX (an elimination half-life of 6.8 h). The single doses of ABT492 and LFX were designed to provide the same AUC/MIC range, from 60 to 480 h. In addition, clinically achievable AUC/MICs were simulated: 870 h with ABT492 and 70 h with LFX. The total antimicrobial effect was expressed by its intensity (IE – area between the control growth and the time-kill/regrowth curves).
Results: The time courses of viable counts that reflect killing and regrowth of *S. aureus* exposed to the quinolones yielded similar patterns. At the AUC/MIC ratios studied, regrowth followed a remarkable reduction in bacterial numbers. However, at a given AUC/MIC ratio, the maximal reductions in the starting inoculum of ABT492-exposed *S. aureus* were greater than with LFX. The times to regrowth were shorter with ABT492 than LFX, because of the relatively rapid fall in ABT492 concentrations to the MIC level, at least at AUC/MICs of 60–480 h. At the higher AUC/MIC ratio (870 h) that is provided by clinical doses of ABT492, regrowth of *S. aureus* was observed much later than with simulation of the clinically achievable AUC/MIC of LFX (70 h). A specific AUC/MIC relationship of IE was inherent in each quinolone. At a given AUC/MIC ratio, the effect of ABT492 was less pronounced than LFX over AUC/MIC ratios from 60 to 480 h. However, at the AUC/MIC of 870 h that corresponds to a 400-mg dose of ABT492, for example, the IE was 2.5 times greater than that of a 500-mg dose of LFX (AUC/MIC 70 h).
Conclusion: These data suggest that ABT492 may be more efficient than LFX against staphylococci at clinically achievable AUC/MIC ratios.

O387 Are AUC/MIC and C_{max}/MIC predicting pharmacological indices for the activity of ciprofloxacin against *Escherichia coli*?

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Objectives: It is common use nowadays to evaluate antibiotics by calculation of pharmacological indices. The International Society for Antimicrobial Pharmacology (ISAP) proposed to use AUC/MIC and C_{max}/MIC values for the prediction of the activity of concentration dependent antibiotics like ciprofloxacin (CIP). By comparison of kill kinetics in batch cultures and in a in vitro model we examined this hypothesis.
Methods: Concentrations which resulted in equal AUC/MIC (0–800) and C_{max}/MIC (0–33.3) were applied in batch cultures and in in vitro models, and kill kinetics of *E. coli* [MIC CIP: 0.03125 mg/L] were performed over 24 h. In the batch culture, different constant CIP concentrations (0.5- to 32-fold MIC) were applied and in the in vitro model *E. coli* was exposed to initial doses of CIP from 1- to 64-fold MIC, that were eliminated with the half-life of 4 h. To compare the antibacterial effect of CIP the areas above the kill curves (AAC) were determined: negative values indicate bacterial growth whereas positive values indicate a bacterial reduction.
Results: The maximal effect in the batch culture and in the in vitro model was observed at a AUC/MIC value of 100. Higher doses (AUC/MIC 200–800) did not show a higher efficacy of CIP on *E. coli*. Within the range of concentration dependency there were no comparable effects of CIP at equal AUC/MIC and C_{max}/MIC values in batch cultures and in the in vitro model (Table 1).

Table 1 Concentration range with differences in the activity of CIP against *E. coli*

AUC/MIC	C_{max}/MIC	batch culture AAC	in vitro model AAC
0	0	−48.08	−44
12.5	0.52	16.26	−51.5
25	1.04	43.51	−31.6
50	2.08	102.9	114.3
100	4.17	120.6	138.9

Conclusions: CIP shows a dose-effect-relationship against *E. coli* only in a narrow range. The hypothesis that AUC/MIC and C_{max}/MIC are predictors for the activity of CIP cannot be generalized because only high AUC/MIC respectively. C_{max}/MIC values, where the maximal effect is already achieved, resulted in similar effect of CIP against *E. coli*. The concentration-time profile is important for the effect of CIP on *E. coli*.

O388 Efficacy and pharmacodynamics of CB-181963 (CAB-175), a new cephalosporin

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Objectives: CB-181963 (formerly CAB-175) is a new cephalosporin with potent activity against a broad spectrum of pathogens including MRSA

(methicillin resistant *S. aureus*). The objective was to determine the efficacy and to quantify the pharmacodynamic parameter predictive of efficacy for CB-181963 against MRSA, *E. coli*, and *K. pneumoniae*.
Methods: A thigh infection model of normal (nonimmunosuppressed) mice was used to investigate the efficacy of CB-181963. Mice were infected by intramuscular inoculation of 1×10^5 to 1×10^7 cfu of MRSA, *E. coli*, or *K. pneumoniae*. Mice were treated subcutaneously with CB-181963, vancomycin, ceftriaxone, or saline control. Dosage was once, twice, or thrice daily for three days starting the day of infection. At 72 h postinfection, thigh tissue was harvested and dilution plated for quantification of bacterial burden. Efficacy was determined based on time over MIC ($T > \text{MIC}$), total drug exposure over MIC (AUC/MIC), and maximal serum concentration over MIC (C_{max}/MIC).
Results: CB-181963 was protective against MRSA, *E. coli*, and *K. pneumoniae* thigh infections in mice. The pharmacodynamic parameter most predictive of efficacy in mice was $T > \text{MIC}$, although AUC/MIC also correlated well in some cases. CB-181963 was effective against MRSA at $T > \text{MIC}$ values equal to 15% of the dosage interval. Efficacy against *E. coli* and *K. pneumoniae* infections required a $T > \text{MIC}$ values of 34% of the dosage interval. Because CB-181963 is more potent against *E. coli* and *K. pneumoniae* (MIC90 = 0.06–0.13 μg/mL) compared to MRSA (MIC90 = 2–4 μg/mL), the overall serum concentrations required to produce effective $T > \text{MIC}$ values are similar for MRSA and Gram-negative infections.
Conclusions: CB-181963 produced efficacy against MRSA and Gram-negative bacterial infections of mouse thigh tissue. The lower $T > \text{MIC}$ values required for efficacy against MRSA compared to *E. coli* or *K. pneumoniae* are an interesting feature of this new cephalosporin. The $T > \text{MIC}$ values required for efficacy (15% for MRSA, 34% for Gram-negative bacteria) warrents further study to determine the clinical potential of this cephalosporin.

O389 Concentration-dependent selection of resistant mutants of *Streptococcus pneumoniae* exposed to moxifloxacin in an in vitro dynamic model: a population analysis

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Objectives: Our recent study with fluoroquinolone-exposed *Staphylococcus aureus*, examined the mutant selection window (MSW) hypothesis, i.e. the concentration range from the MIC to the mutant prevention concentration (MPC), within which it is proposed that resistant mutants are selected. A quinolone-independent relationship of resistance to the ratio of 24-h area under the curve (AUC) to MIC was reflected by a bell shaped curve with a maximum at an AUC/MIC ratio of 43 h. To further examine the MSW concept, *S. pneumoniae* was exposed to moxifloxacin concentrations below the MIC, above the MPC and between the MIC and MPC, i.e. within the MSW.
Methods: Daily administration of moxifloxacin for three days was mimicked using a two-compartment dynamic model with peripheral units containing 10^8 CFU of *S. pneumoniae* ATCC 49 619/ml as a starting inoculum. Changes in *S. pneumoniae* susceptibility were examined by plating the specimens onto moxifloxacin-free agar and agar containing 4× MIC and 8× MIC of moxifloxacin. To compare the effects produced by each dose, the area between the control curve and the time-kill curve (ABBC) was calculated within the first, second and third dosing interval (ABBC-1–3, respectively).
Results: Based on both 4× MIC and 8× MIC data, maximal fs were associated with moxifloxacin concentrations between the MIC and MPC (AUC/MICs 24–47 h) and minimal fs with concentrations below the MIC (AUC/MICs < 10 h) and above the MPC (AUC/MICs > 100 h). A Gaussian-like function fits the AUC/MIC-dependent resistance frequency (f) with the central point at AUC/MIC of 42 h where resistant mutants are selectively enriched, without such selection at AUC/MICs < 10 h and AUC/MICs > 100 h. These findings are consistent with a comparative analysis of the ABBCs: at the lower AUC/MIC ratios (6–24 h) and the higher AUC/MICs (74–224 h) when fs were minimal, ABBC-2 and -3 were similar. At the intermediate AUC/MICs (24–47 h), when fs were maximal, the ABBC-3 was less than the respective ABBC-2.
Conclusions: This study suggests that (i) most pronounced selection of resistant mutants of *S. pneumoniae* and *S. aureus* occurs at similar AUC/MICs of moxifloxacin (ii) there is a strong relation between resistance and the antimicrobial effect of moxifloxacin, and (iii) these findings support the MSW concept.

O390 Pharmacodynamic comparison of linezolid and daptomycin against Gram-positive bacterial infections in cancer patients by Monte Carlo simulation

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Objectives: Pharmacodynamic (PD) indices such as AUC/MIC and time above MIC have been linked to clinical outcome for numerous antibiotics. The AUC/MIC ratio has been found predictive of linezolid (LZD) outcome in animals and in humans (D. Andes, AAC 2002; C. Rayner, ICAAC 2000), and of daptomycin (DAP) in animals (A. Louie, AAC 2001). Our objective was to determine the probability of achieving PD targets with LZD and DAP in our institution.
Methods: Two hundred and fifty-seven Gram-positive blood isolates were collected from hospitalized cancer patients, and MICs determined in duplicate by macrobroth dilution, according to NCCLS standards. Calcium supplementation was utilized in all daptomycin experiments. Resultant MICs and published PK data for LZD and DAP was used to conduct Monte Carlo simulations (MCS) of 5000 patients (Crystal Ball 2000). Target 24-h AUC/MIC ratios were set for LZD at 83 (D. Andes, AAC 2002), and at 517 and 246 for DAP to achieve 80% maximal kill and stasis, respectively (A. Louie, AAC 2001). The probability of achieving these targets was evaluated at the standard doses of 600 mg q12h for LZD and 6 mg/kg q24h for DAP; DAP protein binding was accounted for.
Results: The overall MIC50,90 (range) for LZD and DAP was 2,4 (0.125–64) and 1,4 (0.03–8); there were 119 enterococci (82 vancomycin resistant), 128 staphylococci, and 10 other. The mean (SD) AUC/MIC ratio for LZD was 116.2 (119), with a target attainment probability against these organisms of 63.7%; 51.9% achieved an AUC/MIC ratio of 100. The mean (SD) AUC/MIC ratio for DAP was 1311 (2279), with a target attainment probability of 73.8% for stasis, and 63.4% for 80% maximal kill.
Conclusions: Both LZD and DAP demonstrated activity against pathogens collected from our institution, however, based on this analysis PD target attainment was higher with DAP for stasis endpoints. The somewhat low target attainment rates were primarily a result of higher MICs observed in the enterococci, relative to staphylococci. The LZD results are also in concordance with clinical trial results against VRE, in which approximately 67% cure rates were reported (Zyvox package insert); similar clinical data is not available for DAP. These results also suggest that higher doses and/or combination therapy may be necessary in some patients to achieve optimal cure rates in our institution, especially against VRE.

O391 Intensity of the antimicrobial effect as a tool in pharmacodynamic studies using in vitro models: new aspects

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Objectives: Prediction of the antimicrobial effect (AME) based on its relationships to the MIC-related pharmacokinetic variables, i.e. area under the curve (AUC), peak concentration (C_{max}), etc., is the primary goal of in vitro studies that simulate antibiotic pharmacokinetics. Using endpoints that consider only extent but not duration of AME, the AUC/MIC or C_{max}/MIC relationships may often be established at the AUC/MICs lower than those achieved clinically. At the relatively high AUC/MICs typical for many antibiotic-pathogen pairs, reasonable relationships may be seen using the intensity of AME (IE) that considers both extent and duration of AME.
Methods: To demonstrate the significance of AME duration, the concentration/MIC-time curves that reflect daily dosing of moxifloxacin and levofloxacin at the same AUC/MIC ratio were generated using half-lives of 12 and 6 h, respectively. The accumulation factors were calculated for the specific case when the trough concentration of levofloxacin but not moxifloxacin is equal to the MIC.
Results: Simulations of the quinolone pharmacokinetics predicted a 20% increase of the C_{max}/MIC ratio of moxifloxacin vs. a 2% increase of the C_{max}/MIC of levofloxacin during the treatment. This highlights the impact of unexhausted antimicrobial potential of moxifloxacin's trough concentrations on AME observed at the steady-state conditions. The different AMEs of moxifloxacin and levofloxacin resulted from their different potentials can accurately be reflected by IE but not other endpoints. Based on the IE analysis of quinolone AMEs on *Staphylococcus aureus*, the predicted breakpoints for grepafloxacin and levofloxacin (AUC/MIC 78 h and C_{max}/MIC 13,

respectively) were close to those established in clinical settings (75 h and 12, respectively).
Conclusion: Being the only metric having a physical significance (IE describes the total amount of killed bacteria), IE may be determined at the relatively high AUC/MICs including clinically achievable values. IE shows reasonable AUC/MIC or C_{max}/MIC relationships of AME and allows clinically relevant predictions of AUC/MIC and C_{max}/MIC breakpoints.

O392 Pharmacodynamics of cefepime–aztreonam combination against *Pseudomonas aeruginosa* isolated from cystic fibrosis patients

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Objectives: Previous studies have demonstrated that cefepime (FEP)–aztreonam (ATM) is effective in preventing the emergence of resistance during treatment of *Pseudomonas aeruginosa* in an in vitro pharmacokinetic model (IVPM). ATM was shown to competitively inhibit the AmpC cephalosporinase and enhance the activity of FEP, even against depressed mutants. The study objective was to further evaluate the FEP-ATM combination against clinical isolates of *P. aeruginosa* from cystic fibrosis patients.
Methods: Five strains selected provided a range of susceptibilities from susceptible to high-level resistance. Log-phase cultures ($\sim 10^7 - 10^8$ cfu/mL) were inoculated into the IVPM and exposed to peak concentrations observed in human serum after doses of 1 g of FEP or ATM, or a combinations of each. Drugs were dosed at 0 and 12 h, human pharmacokinetics were simulated and changes in viable counts were measured at 0, 2, 4, 6, 12, and 24 h. Samples removed at 24 h were also plated into agar containing FEP (4× MIC) to detect mutants.
Results: With the susceptible strains, all three regimens provided 2.5–3 logs killing through 6 h. Viable counts increased substantially in FEP-treated cultures and a resistant subpopulation was detected. In contrast, no resistant mutants were detected in cultures treated with ATM or FEP-ATM, and FEP-ATM provided the greatest level of killing over 24 h (3.5–4 logs). Against an intermediate strain, FEP and ATM alone only provided 1–1.5 logs of killing over 24 h, while FEP-ATM provided three logs of killing over 24 h. With one resistant strain, neither FEP or ATM showed any bacterial killing, whereas FEP-ATM decreased viable count approximately one log. With second resistant strain, FEP and ATM decreased viable counts two logs followed by inoculum regrowth. In contrast, no inoculum regrowth was observed with FEP-ATM and viable counts decreased a total of three logs over 24 h.
Conclusions: FEP-ATM provided significant bactericidal activity against four of five strains isolated from cystic fibrosis patients, including one highly resistant strain. Although resistant mutants were selected from two strains with FEP, no resistant mutants were selected with either ATM or FEP-ATM. These data, in combination with previously published data, suggest that FEP-ATM may be an effective combination for the treatment of *P. aeruginosa* infections, and further studies are warranted.

O393 Pivampicillin enhances the activity of ampicillin against intracellular bacteria: studies in a model of J774 macrophages infected with *Listeria monocytogenes*

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Objectives: Pivampicillin, a basic ester prodrug of ampicillin is accumulated in high extent in J774 macrophages (ICAAC 2001–2070, ECCMID 2002: P1349; Chanteux et al. Pharm Res, in press). Since only ampicillin is active against bacteria and ester bond are unstable in biological media, we investigated the ability of PIVA to regenerate intracellular free ampicillin and to be useful to control intracellular infections.
Methods: J774 macrophages were infected with *Listeria monocytogenes* (bacteria to cell ratio 7 : 1) during 1 h for phagocytosis. Cells were washed with PBS and incubated with ampicillin or pivampicillin. At suitable intervals, cells were washed, scraped, lyzed in water, and the lyzates used for determination of viable bacteria by colony counting. Intracellular ampicillin was assayed using an enzymatic method relying on the capacity of the drug to inhibit bacterial D-D-carboxypeptidase (Frère et al. AAC 1980; 18: 506–510).
Results: At an extracellular concentration of 10× the MIC of ampicillin, ampicillin and pivampicillin were mainly bacteriostatic in a 5-h time period. However, when the extracellular concentration was reduced to 0.5× the MIC, only pivampicillin remained bacteriostatic whereas AMPI was inactive. Upon 20 h incubation, pivampicillin lost activity in relation to its hydrolysis in the extracellular milieu (half-life 35 min). Assay for intracellular ampicillin showed a high level of accumulation after 5 h incubation with pivampicillin whereas incubation with free ampicillin showed no drug accumulation (cellular to extracellular ratio 1). This high accumulation decreased over time and at 12 h incubation with pivampicillin, the level of intracellular ampicillin is the same as ampicillin alone. If medium was renewed every 5 h during infection, activity persisted all over the experimental period (20 h).
Conclusions: Pivampicillin is accumulated in macrophages and release large amounts of ampicillin. We suggest that pivampicillin could be a useful drug in the control of intracellular infections.

O394 Pharmacodynamics and potential clinical role of garenoxacin against anaerobic pathogens

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Objectives: Garenoxacin (G) is a new desfluoroquinolone with a broad spectrum of antimicrobial activity including anaerobes such as Bacteroides group organisms (MIC90 $\leq$ 8.0 μg/mL). Moreover, G has been shown to be rapidly accumulated by B. fragilis. Following a 600-mg dose of G, the C_{max} and AUC24 are approximately 10 μg/mL and 100 μg h/mL, respectively (Wise et al. Antimicrob Agents Chemother 2002; 46: 242). Results of time-kill studies found that G was bactericidal at 2× MIC after 48 h against Bacteroides group isolates. In an in vitro pharmacodynamic (PD) model, AUC/MIC ratios $\geq$ 50 provided bactericidal activity and prevention of regrowth in postexposure cultures of Bacteroides group isolates. An analysis of these PD ratios was conducted against *B. fragilis* group organisms based upon human pharmacokinetic parameters.
Methods: PD ratios (C_{max}/MIC, AUC/MIC) for G against B. fragilis group isolates (Snyderman et al. Antimicrob Agents Chemother 2002; 46: 3276) were calculated and are shown in the table.
Results: The peak serum concentration (C_{max}) following a 600-mg dose of G is at least 2× higher than the geometric mean for each group of isolates, and the AUC/MIC ratios are $\geq$50 with the exception of *B. valgatus* (Table).

Organism (no.)	Mean MIC (μg/mL)	C_{max}/MIC	AUC/MIC
B. distasonis (36)	1.8	5.6	56
B. fragilis (288)	0.9	11.1	111
B. ovatus (61)	1.7	5.9	59
B. thetaiotaomicron (136)	1.6	6.25	62
B. uniformis (11)	2.0	4.8	50
B. valgatus (35)	4.2	2.4	24

Conclusion: PD ratios can be attained that would suggest a therapeutic role for G against B. fragilis group isolates. Ongoing clinical trials will determine the viability of G in the treatment of anaerobic infections.

O395 Urine bactericidal activity of gatifloxacin and moxifloxacin against nosocomial uropathogens

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Objectives: The methoxyfluoroquinolones, gatifloxacin (G) and moxifloxacin (M) have similar in vitro activity against uropathogens but significantly different amounts of these drugs are recovered in the urine (G = 73%; M = 20%). In this investigation, we measured urine concentrations and the bactericidal activity of G and M in urine (UBA) against four different uropathogens (nine isolates) by microdilution techniques.
Methods: Eleven normal healthy male volunteers were given a single dose of G (400 mg) followed one week later by a single dose of M (400 mg). Urine samples were collected prior to and at 2, 6, 12, and 24 h after the dose of each drug, and stored at −70°C. Urine levels (HPLC procedure) and cidal titers (NCCLS methodology) were determined at each time period for each subject against isolates of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *E. faecalis*.
Results: Urine concentrations were significantly higher with G. Mean peak (2 h) levels were 250 μg/mL with G and 83 μg/mL with M. Cidal titers in urine were usually higher and more prolonged with G compared to M (Table). This was especially evident against the two strains of *P. aeruginosa*.

MIC (μg/mL) organism	Median duration of UBA (h)			
	G	M	G	M
E. coli	0.06	0.125	24	24
	2.0	2.0	24	12
K. pneumonia	0.125	0.06	24	24
	1.0	2.0	24	6
P. aeruginosa	2.0	4.0	24	2
	4.0	8.0	6	0
E. faecalis	0.5	0.25	24	24
	2.0	2.0	24	24

Conclusion: Both G and M produced prolonged UBA against these common nosocomial uropathogens with the exception of *P. aeruginosa*. In contrast to G, a single dose of M exhibited little to no UBA against the two strains of *P. aeruginosa*.

Fungi: disease, diagnosis and therapy

O396 Caspofungin treatment of invasive candidiasis in cancer patients

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Background: Invasive candidiasis (IC) is often a complication of cancer and its therapy. In a large international study of IC, caspofungin (CAS) was as effective as and better tolerated than amphotericin B (AmB) (NEJM 2002; 25: 2020–9). CAS could provide a therapeutic option for cancer patients (pts) with IC.
Methods: We evaluated cancer pts enrolled in a double-blind randomized trial of CAS (50 mg/day after a 70 mg loading dose) versus AmB (0.6–0.7 for non-neutropenic and 0.7–1.0 mg/kg/day for neutropenic (≤500/μL) pts to treat clinically and microbiologically documented IC. Study design had therapy end 14 days after the last positive culture; pts responding to IV therapy could be switched to oral fluconazole after 10 days. All treated pts with confirmed IC were included in a modified intention to treat (MITT) analysis. A favorable response required complete resolution of signs and symptoms plus documented or presumptive eradication of *Candida*.
Results: Seventy-four of 224 (33%) pts in the MITT population had active malignancies (30 hematologic and 44 solid tumors). Twenty-five (83%) hematologic cancers were acute or chronic leukemia. Twenty-two (50%) solid tumors were related to the GI tract: 14 colon, four gastric, four esophageal. Other common sites ($n > 2$) included six pancreatic, three bladder, and three gynecologic. Pts with hematologic cancer were younger than those with solid tumors (median (range) age: 49 (19–74) vs. 59 (19–81) years) and had higher baseline APACHE II scores (median (range): 20 (0–28) vs. 14 (5–35)). Neutropenia was present on entry in 23 (77%) pts with hematologic cancer (22 had leukemia) and in one (3%) pt with a solid tumor. Candidemia was demonstrated in 29 (97%) and 36 (82%) pts with hematologic and solid organ cancers. Most commonly isolated *Candida* spp. are shown in Table 1. Table 2 shows response rates at the end of study therapy. CAS- (7/14) and AmB-treated (4/10) neutropenic pts responded favorably. CAS (3/10) and AmB (3/7) pts with profound neutropenia at baseline (<100/μL) had favorable responses. CAS (4/6) and AmB (4/7) pts whose neutropenia resolved responded, compared to 3/8 CAS and 0/3 AmB pts with persistent neutropenia.

Table 1

	C. albicans	*C. tropicalis*	*C. parapsilosis*	*C. glabrata*	*C. krusei*
Cancer (76 isolates)	31 (41%)	15 (20)%	12 (16%)	8 (11%)	5 (7%)
Hematologic (27)	8 (30%)	9 (33%)	3 (11%)	0 (0%)	4 (15%)
Solid tumors (49)	23 (47%)	6 (12%)	9 (18%)	8 (16%)	1 (2%)

Table 2

	CAS	AmB	Total
Cancer (74 patients)	23/33 (70%)	23/41 (56%)	46/74 (62%)
Hematologic (30)	11/18 (61%)	6/12 (50%)	17/30 (57%)
Solid tumors (44)	12/15 (80%)	17/29 (59%)	29/44 (66%)

Conclusions: Less than 30% of pts in our IC study had underlying cancer, usually leukemia or GI tumors. Although *C. albicans* was the single most frequent isolate in cancer pts, >50% of cases were due to nonalbicans spp. Response rates were lower for neutropenic than non-neutropenic pts. Overall, 70% of cancer pts had a favorable response to CAS, when only 56% of pts had such a response to AmB.

O397 Radiologic findings in acute invasive pulmonary aspergillosis: Utility and reliability of halo sign and air-crescent sign for diagnosis and treatment of IPA in high-risk patients

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Objectives: To characterize initial imaging findings in acute invasive pulmonary aspergillosis (IPA); to evaluate utility and reliability of the halo sign (HS) and air-crescent sign (ACS) for diagnosis and treatment of IPA in high-risk patients.
Methods: Baseline imaging and/or mycology and treatment response (TR) were reviewed for 343 patients who participated in a recent prospective comparative aspergillosis treatment study. Baseline HS or ACS, confirmed by an independent data review committee (DRC), met criteria for IPA in patients with hematopoietic stem cell transplant (HSCT) or hematologic condition (HC) with neutropenia. Independent diagnoses of IPA by DRC radiologists, based on standard definitions, were compared with those of site investigators.
Results: In 254 patients with DRC-confirmed baseline diagnosis of IPA, 141/254 (56%) had at least one nodular lesion with HS and 15/254 (6%) had ACS. One hundred and thirty-eight of 254 (54%) patients had both mycologic and CT data at baseline: 59/138 (43%) had either HS or ACS (48 had HS, 11 had ACS). Seventy-nine patients had baseline diagnosis of IPA based on mycology alone (without HS or ACS on CT): 66/79 (84%) had nodular lesions including 41 with unsharp margination (UM). In the 13 remaining patients, seven had consolidations, two had centrilobular opacities, and one had each of the following: cavitary lesion, non-nodular infarct (NNI), ground-glass opacity (GGO), pleural lesion. TR was satisfactory in 79/154 (51%) patients with HS or ACS on CT, and was satisfactory in 51/95 (54%) with HS or ACS on CT without supporting mycology. In contrast, TR was satisfactory in only 30/98 (31%) with a baseline diagnosis of IPA based on mycology alone. HS or ACS was confirmed by the DRC in 95/148 (64%) of patients entered into the study by the site investigator with a diagnosis of IPA based on CT alone. The major radiologic findings in the 53 nonconfirmed cases included nodular lesions in 48/53 (91%), with UM in 39.
Conclusions: Nodular lesions with HS and ACS dominated baseline radiologic findings in these patients with IPA. The TR data support a hypothesis that patients at risk of IPA presenting with nodular lesions with HS or ACS benefit from antifungal therapy even with absent mycologic findings. Nonreproducibility of HS may result from difficulty in differentiation of HS from UM.

O398 Rhodanine-3-acetic acid derivatives as potent inhibitors of fungal protein: mannosyl transferase 1 (PMT1)

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The incidence of life-threatening fungal infections has multiplied dramatically as the population of immunocompromised individuals has increased. Present therapeutic options are limited to three main classes of compound: polyenes, azoles, and candins. The utility of polyenes is limited by nephrotoxicity, and resistance is emerging to azoles. There is, therefore, a need for new antifungal compounds with novel modes of action for use in treating or preventing such fungal infections. *O*-linked mannoproteins constitute a significant component of the fungal cell wall of *Candida* and other pathogenic fungi, and are believed to confer the cell surface properties involved in adhesion and host interactions. The key step in the biosynthesis of these mannoproteins is catalyzed by a family of protein:mannosyl transferases (PMTs). Deletion of the PMT1 gene from *Candida albicans* results in a strain that is no longer virulent in animal models. The strain also shows several phenotypes associated with cell wall defects. A library of 50 000 compounds was screened against PMT1, and a series of weak inhibitors (IC_{50} *c.* 50 mmol) were identified. The synthesis of analogs of these inhibitors resulted in the discovery of compounds with $IC_{50} < 200$ nmol, and good oral bioavailability. PMT1 inhibitors confer the phenotypes of the pmt1 knockout strain upon treated cells. These phenotypes include clumping, increased sensitivity to aminoglycosides, failure to form pseudo-hyphae in response to a range of stimuli, reduction in chitinase, and reduced adhesion. The compounds are also active against other members of the PMT family of enzymes. The range of phenotypes reflects the many functions of the mannoproteins, however, it is not clear which is responsible for the loss of virulence in vivo. The compounds are active against many other fungi, suggesting that PMT1 function is conserved across a range of fungal species. Data demonstrating the activity of these compounds in vitro and in vivo will be presented.

O399 A proteomics approach to study virulence in the pathogenic fungus *Aspergillus fumigatus*

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Aspergillus sp. are saprophytic fungi, which are found in a wide variety of environmental niches. *Aspergillus fumigatus* is the most readily associated with human infection, with other species being identified as causative agents in some cases (*A. niger*, *A. nidulans*, *A. terreus,* and *A. flavus*). The average human may inhale several hundred conidia per day. In a healthy individual, these will be dealt with by innate immune mechanisms and cause no infection. However, if an immunosuppressed individual becomes exposed to *Aspergillus* conidia and an infection occurs which persists, the outcome is often fatal. There are several factors that may be involved in the virulence of *A. fumigatus* including pigments, adhesins, toxic molecules, and enzymes. A proteomics-based approach has been taken to help identify proteins which may be associated with the virulence of *A. fumigatus*. Spores harvested from *A. fumigatus* were inoculated into a cell line of human epithelial lung cells (HEL) grown in cell culture media (CCM) (virulent model) and into CCM only (avirulent model). Proteins were extracted at days 4 and 5 from the virulent model, when RT-PCR had indicated expression of various putative virulence factors, and from the avirulent model at day 5 which served as a control. Samples were analyzed following two-dimensional (2-D) gel electrophoresis. A number of differentially expressed proteins were detected with the protein profiles of days 4 and 5 being significantly different from the control. These proteins were subject to MALDI-ToF MS (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) analysis to elucidate their identity. Preliminary analysis has shown some similarity to a fatty acid synthetase from *A. parasiticus* and a DNA topoisomerase II from *A. ochraceus* and *A. flavus*.

O400 An open phase II study of the efficacy of micafungin (FK463) alone and in combination for the treatment of invasive aspergillosis (IA) in adults and children

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Objectives: To assess the efficacy of micafungin as primary and salvage therapy of IA, alone and in combination with other antifungal drugs.
Methods: Patients (pts) with probable or proven IA failing, likely to fail or intolerant of $\geq$3 days licensed antifungal therapy were enrolled in a multinational phase II study. Micafungin was substituted for (monotherapy group), or added to therapy (combination group) and given once daily at a starting dose of 75 mg/day or 1.5 mg/kg if $\leq$40 kg. Dose escalation was allowed. Response was assessed clinically, radiologically, and mycologically and recorded as complete, partial, stable or failure. All enrolments were reviewed by an expert panel. Per protocol analysis included pts who received $\geq$7 days of micafungin and had confirmed IA on review (PP population). A separate analysis of PP population who failed $\geq$7 days of prior antifungal therapy at full therapeutic doses (salvage group) was done.
Results: Two hundred and eighty-three pts were enrolled, aged from 9 weeks to 84 years (mean 37 years); 63 (22.3%) were <16 years, 181 (64%) were male, 81 (29%) neutropenic ($<500/mm^3$). One hundred and four (36%) completed therapy and 165 (57%) died. 60% of adults had a dose escalation, and 25% received $\geq$200 mg/day. Median duration of dosing was 34 days in adults and 37 days in children. A total of 179 were in the PP population. Response (CR + PR) was seen in 27/38 (45%) in monotherapy group, and 50/141 (35%) in the combination group. Allogeneic HSCT pts (19/70, 27%) and leukemic pts (32/64, 50%) responded. One hundred and twenty pts were in the salvage group; 115 received combination therapy. Response (CR + PR) was seen in 39/120 (33%), 11/49 (22%) in allogeneic HSCT pts, and 22/45 (49%) in leukemic pts. Responses were seen for all *Aspergillus* spp., except *A. terreus* (0/7). Few attributable SAEs were observed.
Conclusions: Micafungin is effective in the treatment of IA, alone or in combination, and carries very few adverse reactions.

O401 Real-time PCR is superior to galactomannan and mannan ELISA for the early detection of invasive fungal infection in hematology patients

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Invasive fungal infections (IFI) are an increasingly important cause of morbidity and mortality in patients with hematologic malignancy and stem cell transplantation, yet remain difficult to diagnose. We have designed a prospective, blinded study to compare screening with a real-time, pan-fungal PCR assay using the LightCycler technique with ELISA for galactomannan (GM) and mannan (M) for the early detection of IFI in immunocompromised hematology patients. Between December 2000 and December 2001, 133 patients (204 treatment episodes) were recruited. On admission, and twice weekly, clotted and EDTA blood samples were collected during each treatment episode and analyzed using PCR to detect 18S ribosomal fungal DNA and the Platelia *Aspergillus* and *Candida* ELISA. There were 22 cases of EORTC defined IFI: 3 proven, 9 probable and 10 possible. The sensitivity of the PCR was 10 CFU/mL for *Candida* DNA and 20 CFU/mL for *Aspergillus* DNA. Positive PCR results were recorded in 84.1% of patients (range 1–14 samples). In 50/204 (24.5%) treatment episodes, PCR was sequentially positive. In a further 84/204 (41.2%) treatment episodes, PCR was positive either on a single occasion (52/204, 25.5%) or intermittently

(32/204, 15.7%). Of the 50 episodes with sequential PCR positivity, 14 were graded as EORTC proven (3/3 episodes), probable (7/9 episodes), or possible (4/10 episodes). In 9 of these 14 episodes, sequential PCR positivity was documented between 1 and 71 days (mean 28, median 27) prior to commencement of empiric antifungal therapy. Sequential PCR positivity was also detected in 36 episodes that were EORTC negative. In 10, sequential PCR positivity resolved with neutrophil recovery; in 11 whilst still neutropenic; and in a further 5, the patients developed IFI in subsequent episodes. The negative predictive value of a negative or single/intermittent positive result for proven/probable IFI was 98.7%. Of 82 episodes analyzed with the M ELISA, 1 sequential and 7 single positives were recorded, all with no evidence of IFI. Positive GM ELISA results were recorded in 22/204 episodes; 1 was sequential positive and had proven IA while in a case with a single positive there was evidence of probable IA. In 183 episodes with negative GM ELISA results, there were 19 cases of IFI. These results provide evidence that prospective screening using PCR is a useful diagnostic tool for early detection of IFI and is superior to GM and M ELISA.

O402 Identification of novel cell surface proteins in *Candida albicans* and investigation of their role in adhesion and virulence

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Objectives: *Candida albicans* is responsible for the majority of hospital-based fungal infections and is a particularly important pathogen in immunocompromised patients, where it can cause superficial mucocutaneous infections as well as life-threatening systemic infections. We are seeking new ways to identify *C. albicans* cell surface proteins involved in adhesion (an early step in pathogenesis) or virulence.
Methods and results: Towards that end, we developed a computational biology approach and identified 210 potential cell surface proteins. Among the pool of 210 candidates that fulfilled the bioinformatics criteria, we selected a subset of 6 proteins (named CSF1–6 for cell surface factors) for further biologic characterization. We first showed that all CSF genes are expressed by RT-PCR and then disrupted both alleles using a two-step gene disruption method. We further investigated the effect of the loss of each CSF gene on cell wall integrity, adhesion to mammalian cells, and virulence in an animal infection model. As a result, we identified two adhesins and four additional factors that are important for cell wall integrity and virulence.
Conclusion: Assuming that all six CSF proteins are major *Candida* cell surface proteins, they represent promising targets for the development of new antifungal drugs, therapeutic antibodies, and/or vaccines.

O403 A phase 2 dose-ranging study of the safety and efficacy of anidulafungin in invasive candidiasis

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Introduction: Anidulafungin (VER002) is a novel cyclic lipopeptide antifungal agent of the echinocandin class. In vitro, anidulafungin is highly active against *Candida* and other fungal pathogens. We have previously shown that steady state is rapidly achieved with a loading dose. The pharmacokinetic profile and antimicrobial spectrum of anidulafungin suggest that it may be useful in serious fungal infections, including invasive candidiasis (IC).
Objectives: To evaluate the clinical and microbiologic efficacy and safety of three dose regimens of anidulafungin in patients with IC.
Methods: Adult patients (pts) at least 18 years of age with documented IC, including candidemia or histologic or culture evidence of infection in a normally sterile site, and expected survival >72 h, were randomized to one of three dose regimens: Arm A: 100 mg intravenous (i.v.) loading dose on Day 1 followed by 50 mg IV daily (100/50 mg); Arm B: 150/75 mg; Arm C: 200/100 mg. Pts received treatment for 2 weeks beyond cure or improvement, up to 42 days. A test of cure (TOC) visit occurred 2 weeks after end of therapy (EOT). Safety evaluations included standard lab assessments and recording of adverse events (AEs). The primary efficacy analysis was global response in the evaluable population at TOC. A successful global response required a successful clinical and microbiologic outcome.
Results: One hundred and twenty-three pts were enrolled, 120 (40 in each arm) received at least one dose of anidulafungin. At baseline, the most common species was *Candida albicans*, accounting for about half of all isolates, followed by *C. glabrata*. Mean APACHE II score was 15.6. The overall mean duration of therapy was 17 days. There was a trend to greater response in the higher dose arms. The number and proportion of pts with a successful global response in the evaluable population at EOT and TOC are shown in the Table 1.

Table 1

Group time point	100/50 mg, *n/N* (%)	150/75 mg, *n/N* (%)	200/100 mg, *n/N* (%)
EOT	21/26 (81)	25/28 (89)	23/26 (88)
TOC	13/19 (68)	19/23 (83)	19/23 (83)

AEs, including serious AEs, and laboratory safety data did not differ by group. Per pathogen responses were similar among all species.
Conclusion: Anidulafungin appears effective in the treatment of IC. Higher doses are as well tolerated as the lowest dose regimen, and may provide superior efficacy. A definitive Phase 3 trial is underway.

O404 Correlation between Sensititre-YeastOne® method and the proposed procedure for antifungal susceptibility testing of the European Committee on Antibiotic Susceptibility Testing

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Objectives: The European Committee on Antibiotic Susceptibility Testing (EUCAST) has developed a proposed standard for antifungal susceptibility testing of yeasts based on reference procedure of NCCLS M27-A, but incorporating modifications (RPMI-2% glucose, inoculum size of 105 CFU/mL, spectrophotometrical reading) in order to get an automated AFST and to shorten the incubation period for MIC determination from 48 to 24 h. We have analyzed the correlation between EUCAST procedure and the commercial method Sensititre-YeastOne, a microplate-based procedure for in vitro testing of amphotericin B (AB), fluconazole (FLZ), itraconazole (ITZ), ketoconazole (KTZ), and flucytosine (FC).
Methods: A total of 60 *Candida* spp. clinical isolates (10 each *C. albicans* (CA), *C. tropicalis* (CT), *C. parapsilosis* (CP), *C. glabrata* (CG), *C. krusei* (CK), *C. lusitaniae* (CL)) were tested. CK ATCC6258 and CP ATCC22019 were included as QC strains. Triplicate testing on three separate days was performed.
Analysis: (i) Correlation: intraclass correlation coefficient (ICC), over a maximum value of 1; (ii) agreement (AGR): discrepancies of no more than two 2× dilutions.
Results: Overall, the correlation was 0.92 and the AGR was 75%. By species, the poorest correlation values were obtained for CT isolates, which exhibited a percentage of AGR of 56%. By antifungal agents, the lowest correlation values were obtained for AB, with ICC and AGR values of 0.29 and 50.5%, respectively. The correlation for azole resistant isolates were excellent. The Sensititre-YeastOne MICs of FLZ and ITZ for azole resistant strains were >32 and >0.5 mg/L, respectively.
Conclusions: (1) The correlation between EUCAST procedure and Sensititre-YeatsOne method is high with average correlation values of 0.92 and AGR percentages of 75%.
(2) The methods are not comparable for AB.
(3) Sensititre-YeastOne method detects azole resistant strains.

O405 High-risk patients, candidemia and antifungal susceptibility profile: 7-year clinical audit in four high-risk units of a tertiary care hospital

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Introduction: Significant morbidity and mortality is associated with opportunistic fungal infections in immunocompromised patients. An audit of antifungal susceptibility profiles of candidemic isolates obtained from patients in high-risk units (hematology, nephrology, oncology and intensive trauma unit) of the Western Infirmary and Gartnavel General Hospital, Glasgow, was carried out over a 7-year period (1996–2002).

Objective: The aim was to establish baseline data of the prevalence of different *Candida* species in fungemic episodes in the four high-risk units of the hospitals and correlation with their antifungal susceptibility profile.
Methods: Data on yeast isolates and antifungal susceptibility profile in blood cultures from patients in these units were audited.
Results: Forty-one isolates were obtained from 52 episodes of candidemia from 40 patients. *Candida albicans* (51.2%) was the commonest isolate overall, followed by *C. parapsilosis* (24.4%), and *C. glabrata* (14.6%). In oncology units: *C. parapsilosis* accounted for 50% (8/16) isolates, followed by *C. albicans* in 31.25% (5/16), while in hematology units *C. parapsilosis* and *C. albicans* accounted for 33.3% (2/6) isolates each. *C. glabrata* closely followed in incidence in all the four areas. All isolates demonstrated in vitro susceptibility to amphotericin B. Susceptibility to Fluconazole and Itraconazole was 85.7 and 95.3%, respectively, for *C. albicans*, 80% for both agents for *C. parapsilosis*, 0 and 16.7%, respectively, for *C. glabrata*, 0% for both agents for *C. krusei*. Distribution of the different candida species and varying sensitivity profiles is discussed.
Conclusion: *Candida albicans* was the commonest yeast causing candidemia. However, the high prevalence of *Candida* species other than *C. albicans* in 48.8% of isolates is striking. *C. parapsilosis* was the commonest isolate in the oncology units and use of total parental nutrition (TPN) in these patients may be a factor. Results of antifungal susceptibility testing (AFST) justify use of amphotericin B as empirical choice in candidemia pending identification and AFST. This work is a useful yardstick to prospectively compare the efficacy of newer azoles and echinocandins with current antifungal agents.

Staphylococcal and catheter associated hospital-acquired infections

P406 An extended-source outbreak of *Staphylococcus epidermidis* infections among patients undergoing cardiac surgery

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Objectives: To describe the first *Staphylococcus epidermidis* outbreak detected among cardiac surgery patients in our new Hospital, to identify the cause and/or source of the outbreak and to prevent the occurrence of new cases.
Methods: Design, retrospective cohort study. Setting, a 260-bed community referral center. Probable case definition, valve surgery patient operated in Hospital de la Ribera (HR) with clinical diagnosis of endocarditis and/or mediastinitis from January 2002 to June 2002. Study population, patients undergoing valve surgery in HR from January 2000 to June 2002. *S. epidermidis* were identified by MicroScan Pos panel (Dade MicroScan). DNA typing with Pulsed Field Gel Electrophoresis was not performed.
Results: From January to March 2002 several cases of mediastinitis and endocarditis among patients undergoing cardiac surgery were detected. In 2000, there was no case detected. During the first 4 months of 2001, incidence of infection was 5.1% (2/39). On the same time frame in 2002 incidence was 11.7% (4/34). There were no alternative explanations for this increase such as changes in nosocomial infection surveillance, preoperative, intraoperative or postoperative procedures. Four additional cases were detected, one in May and three in June. Overall, eight patients developed infection including four with mediastinitis and endocarditis, three with mediastinitis and one with endocarditis. The epidemic curve suggested an extended-source outbreak. There were no statistical differences between cases and controls with respect to age, gender, preoperative hospital stay, antimicrobial prophylaxis, time on bypass, central venous catheter duration, NNIS risk index or ICU stay. Patients with underlying illness such as COPD were 5.3 times more likely to become cases (95% CI 1.4–20.3). Mean duration of surgery was higher in non cases 162.4 (57.8) vs. 123.8 (23.7) (SD, min), $P = 0.002$. All of the infections occurred in patients following aortic valve replacement, $P = 0.02$. Of all staff evaluated there were no associations with infection. Environmental investigations did not provide additional information.
Conclusions: There was no point-source associated with infection. The cause of this outbreak was likely multifactorial. Changes suggested during the investigation included improvement of patient skin preparation, preoperative hand/forearm antisepsis, antimicrobial prophylaxis and surgical attire which contributed to the resolution of the outbreak.

P407 Nosocomial bloodstream infection with multiple location due to multiresistant *Staphylococcus hemolyticus*

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Introduction: Nosocomial infections, and especially bacteremia, are serious causes of morbidity and mortality, especially when present multiresistant microorganisms. This report describes a case of sepsis due to *Staphylococcus haemolyticus* with multiple location.
Methods and Results: Results: A 76-year-old woman was admitted because of fever, respiratory distress, tachycardia, anemia and fatigue. Ten weeks ago, she underwent a laparoscopic bile resection, complicated by pneumonia and liver abscess which was drained surgically. Her past medical history included seizures under antiepileptic treatment, possibly due to cysticercosis. The admission laboratory tests revealed WBCs 26800/mL × 103 (82% neutrophils), Ht 29.2%, ferritin 685 ng/mL, elevated transaminase levels and ESR 111 mm/1 h, while blood pressure was 80/40 mmHg. A central venous catheter was used as a well as a vesical one. Two sets of blood cultures and a urine one were taken and piperacillin–tazobactam (4.5 mg × 4, i.v.) plus amikacin (500 mg × 2, i.v.) were administered. On day 10, two new sets of blood cultures because of fever 40°C were taken. Blood cultures were carried out by BACTEC 9050 system (Becton Dickinson). The venous catheter was changed and cultured, but the cultures were negative. Transesophageal echocardiogram revealed aortic valve vegetation. Three bottles of blood cultures and a urine one grew *Staphylococcus haemolyticus*. Species identification was performed by Gram-stain, catalase test(+) and coagulase test by Staphytect Plus system-Oxoid(–) and APISTAPH (Biomerieux). Susceptibility test was performed by disc diffusion method (Kirby-Bauer in Muller Hinton agar). The strain was resistant to penicillin, gentamycin, amikacin, piperacillin–tazobactam ciprofloxacin, clindamycin, imipenem and but susceptible to vancomycin, rifampicin netilmicine and teicoplanin. *Pseudomonas* spp. was isolated from a skin lesion culture. The therapy was replaced by vancomycin (500 mg × 4, i.v.) plus rifampicin (300 mg × 3, i.v.) that were administered to the patient for 4 weeks. The patient on day 17 was afebrile and she exited the hospital, with clinical and laboratory improvement.
Conclusions: Though nosocomial infections have a high mortality rate frequent blood cultures enable accurate diagnosis and optimal treatment in patients with risk factors (catheters, previous long-term hospitalization) and multiresistant nosocomial strains.

P408 Incidence and clinical significance of *Staphylococcus lugdunensis*: a prospective microbiological and clinical study

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Objectives: To evaluate the incidence and clinical significance of *Staphylococcus lugdunensis* in a teaching general hospital over a 4-month period.
Methods: From September 2002 (when we started a routine protocol searching for *S. lugdunensis* in our hospital) to December 2002, all *S. lugdunensis* recovered in our microbiology laboratory were studied. The isolates were identified using the MicroScan system and confirmed by an additional positive ornithine decarboxylase test. Susceptibility testing was performed by the broth microdilution method using the MicroScan system and beta-lactamase production was detected using the nitrocephin test. All patients from whom *S. lugdunensis* was isolated were prospectively followed up.
Results: Over the study period a total of 906 coagulase-negative staphylococci were isolated. Among those, 19 isolates (2%) were *S. lugdunensis*. All but two were beta-lactamase negative, all were methicillin-susceptible and susceptible to multiple antimicrobials. The origins of the 19 isolates were wound (12), joint fluid (2), umbilical (1), abscess (1), CSF (1), IV catheter (1), and ear fluid (1) and corresponded to 17 patients hospitalized in 11 different wards. Eight patients (47%) were infected and nine were colonized. Among the infected patients (62% females, median age 73 years; seven adults and one neonate), seven (87.5%) presented underlying diseases (four organic, two tumoral and

one HIV+). In five cases *S. lugdunensis* was nosocomially acquired. Infections were: wound infection (4), omphalitis (1), renal abscess (1), prosthetic joint infection (1), and catheter-related infection (1). The infection was polymicrobial in four cases (50%). Seven patients recovered with adequate treatment. One patient died but mortality was not attributable to *S. lugdunensis* infection.
Conclusions: In our hospital *S. lugdunensis* represents 2% of all coagulase-negative staphylococci isolates, and this microorganism is susceptible to multiple antimicrobials. Only half of the isolates of *S. lugdunensis* have clinical significance, and infections due to *S. lugdunensis* present good evolution with adequate antimicrobial treatment. The isolation of *S. lugdunensis* should not be discarded as a contaminant without careful consideration.

P409 Does enteritis due to methicillin-resistant *Staphylococcus aureus* actually exist? – Clinical and animal study

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Objective: Although *Staphylococcus aureus* was considered to be responsible for antibiotic-associated colitis, the role of *S. aureus* is now the subject of debate. The present study was performed in order to determine whether methicillin-resistant *Staphylococcus aureus* (MRSA) is capable of causing enteritis and whether recovery of MRSA from stool specimens originated from patients with diarrhea implies MRSA enteritis.
Methods: Clinical study – we reviewed 163 patients diagnosed with MRSA enteritis between 1992 and 2001 in a teaching hospital in Japan and 34 case reports of MRSA enteritis published in medical journals since 1990. Of the 197 cases, 21 cases were selected using our criteria. These criteria were as follows: (1) presence of diarrhea (2) subsequent dominant or single recovery of MRSA from stool specimens (3) negative results of toxins and cultures of *Clostridium difficile* (4) obvious improvement after treatment with vancomycin. Animal study – we adapted the streptomycin-treated murine model of chronic mucosal colonization. IC mice first received 1 mg of streptomycin per mL of drinking water for 5 days and then orally ingested 107 CFU/mL H_2O of MRSA (±streptomycin) for 1–5 days and stool cultures were performed periodically.
Results: The average age of the 21 patients (11 males, 10 females) suggested to have MRSA enteritis, was 61.4 years. Most of the 21 patients showed fever and abnormal white blood cell counts. Nineteen patients had previously been prescribed more than one antibiotic before diarrhea occurred. Although the antibiotics tended to be broad-spectrum, relationships between specific antibiotics and occurrence of enteritis were not observed. Two patients died of multiple organ failure. In animal models, chronic colonization with MRSA for up to 10 weeks was determined by fecal culture. Stools of MRSA-colonized mouse became soft but not diarrheal. A single day of MRSA ingestion with no combined use of antibiotics was sufficient for enteric colonization with MRSA.
Conclusion: Although diarrheal diseases, of which the cause is strongly suggestive of MRSA, actually exist, the clinical courses in the present study were varied. Moreover, MRSA easily colonizes in the gastrointestinal mucosa and colonization is sustained for long periods. Mere recovery of MRSA from stool specimens does not indicate MRSA enteritis and other causative factors may be involved.

P410 The isolation of GISA following prolonged glycopeptide therapy

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Objectives: The description of the isolation of a glycopeptide intermediate-resistant *S. aureus* (GISA) following glycopeptide therapy.
Methods: Case study.
Results: A patient who developed post operative complications was admitted to our ITU. She developed an MRSA bacteremia that was treated with vancomycin. During this period she developed tricuspid endocarditis, and required an combination of glycopeptide antibiotics (plus rifampicin) for a total of 75 days. The MIC to vancomycin of her MRSA increased from 0.5 to 16 mg/L, and to teicoplanin from <4 to 16 mg/L, resulting in treatment failure and death. A series of Infection Control measures were put in place.
Conclusions: In this case a GISA arose from the prolonged (75 days) use of glycopeptide antibiotics, to treat a tricuspid endocarditis, resulting in treatment failure.

P411 Rate of carriage of MRSA in children undergoing cardiac surgery

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is recognized as a major cause of Hospital Acquired infection in the UK. Surgical wound infection is one of the most frequent MRSA-induced infections with often disastrous consequences. In order to reduce the risk of MRSA infection we have introduced screening prior to cardiac surgery and are now able to evaluate the rate of MRSA colonization in these patients.
Methods: Three hundred and forty nine patients were admitted to the unit between August 2001 and December 2002, 191 were male and 158 were female. Of these 321 patients admitted for cardiac surgery had nasal, throat swabs and swabs of any pre-existing wound sites cultured.
Results: Of 321 patients, 12 were found to be colonized with MRSA. Five were positive on nasal swabs and multiple sites, four on throat swabs alone. The overall positivity rate was 3.7%.
Discussion and conclusions: Anecdotally it is thought that MRSA colonization/infection is less common in the pediatric setting. However, a study in Nashville showed that 1.2% (6 out of 500) of children at well-child visits at either a university hospital pediatric clinic or a private pediatric office were positive for MRSA. This was supported by another study in which 0.6% (3 out of 500) healthy children less than 16 years of age attending for well child care in Chicago were again positive for MRSA. However, at present little is known about MRSA carriage rates amongst children in the UK. A recent UK study in which adult patients admitted to general surgical and orthopedic ward were studied for MRSA colonization revealed an MRSA prevalence of 5.3% of whom 65% were nasal carriers and 35% had MRSA colonization elsewhere. The rate of 3.7% found in pediatric patients is lower than that found in adult patients screened prior to surgery but is higher than the prevalence in the general adult population in the UK. Little is known of the incidence of MRSA in pediatric populations but the organism appears more widespread in children who are about to undergo cardiac surgery than in the general adult population. From the point of view of effective screening four of the MRSA positive patients were identified on throat swab alone. The fact that throat and perianal site swabs have a higher sensitivity in identifying children colonized with MRSA than nasal swabs alone is similar to the findings in a previous Canadian study in children and has important implications for screening pediatric populations.

P412 Linezolid: in vitro activity and clinical effectiveness in cardiac surgery intensive care unit patients

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Objective: To study in vitro sensitivity of MRS CN and *Enterococcus* spp. to Linezolid. To assess its clinical effectiveness in cardiac surgery patients with various nosocomial infections caused by troublesome gr + cocci.
Methods: I stage (in vitro): 90 sequential strains of gr + cocci (MRS CN $n = 60$, *Enterococcus* spp. $n = 30$), cultivated in microbiology laboratory from blood ($n = 45$), sputum ($n = 32$), urine ($n = 5$), wounds ($n = 5$) and cardiac valves ($n = 3$). Following NCCLS recommendations diffuse disk test were used to assess bacterial sensitivity. All strains (100%) of MRS CN and *Enterococci* spp. were sensitive to linezolid in vitro, dependless their sensitivity to other antibiotics. II stage (clinical): 10 patients, including pediatric ($n = 3$, 3 months-12 years) and adult ($n = 7$, 17–65 years) with nosocomial infections caused by multiresistant gr + cocci. Infective endocarditis ($n = 4$), pneumonia ($n = 2$), wound infection ($n = 3$), sepsis ($n = 1$). MRS was cultivated from samples in all cases. Glycopeptides could not be administered in six cases due to: intolerability of vancomycin ($n = 1$), kidney failure ($n = 1$), vamcomycin ineffectiveness (four cases). Linezolide was administered to all patients per os (tablets or suspension): 600 mg twice a day to adult patients and daily dose of 10 mg/kg in two intakes for pediatric patients. The treatment course was 5 days in three cases and 10 days in seven cases. Monotherapy with linezolide administered in one case and combined therapy with other antibiotics in the other nine. Symptoms decrease including fall of temperature and positive dynamics in hemogramm started from the fourth day of therapy. No toxic or other side-effects were registered.
Conclusions:
1. Linezolide effectiveness against MRS CN is comparable with that of vancomycin.

2. Linezolide is effective against all *Enterococci* dependless their sensitivity/resistance to other antibiotics.
3. Linezolide is an optimal alternative to vancomycin, especially in cases when vancomycin is contraindicated.
4. Absence of nefrotoxicity allows to recommend Linezolid as a drug of choice in cases of kidney failure and multiorgan failure.

P413 Colonization with vancomycin-resistant Enterococci among hemodialysis patients in Greece

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Objective: Sparse data are available on infection or colonization with Vancomycin-Resistant Enterococci (VRE) in Greece. The purpose of this study was to evaluate the incidence of VRE colonization among patients treated in Renal Dialysis Units located in the same region and characterize strains by phenotypical and genotypical methods.
Materials and methods: During a 4-month period (1/9–30/12/01) rectal swabs or fecal specimens were obtained from 334 consenting patients and were plated on Enterococossel agar (Becton Dickinson) with 6 mg/L vancomycin (Elli-Lilly). Patients were treated in four Dialysis Units (Units I to IV) located in Western Attica. Unit I is located within the major hospital of this area, which serves as a referral center for these patients. Demographical data were collected by a questionnaire. Isolates were identified to the genus level by standard methods and speciated by ATB (BioMerieux) and a multiplex PCR assay. Susceptibility testing was performed by Vitek (BioMerieux) and confirmed by *E*-test (AB Biodisk) and PCR. DNA fingerprinting of VRE isolates was performed by Pulsed Field Gel Electrophoresis (PFGE).
Results: Thirteen *Enterococcus faecalis* strains were identified by PCR (incidence 3.9%, range among Units 3–4.8%), which were vancomycin resistant by Vitek and *E*-test and possessed the *vanA* gene. Resistance to ampicillin, erythromycin, ciprofloxacin, vancomycin, teicoplanin and streptomycin was the predominant phenotype (8 out of 13 isolates, 62%). DNA fingerprinting allocated the 13 VRE isolates into six different types designated A (two strains), B (six strains), C (two subtype strains) and D to F (one strain each). Unit IV demonstrated two VRE clusters consisting of type A and type B isolates (two and four strains, respectively). Type B isolates were shared by three Units: Unit II (one strain), Unit III (one strain) and Unit IV (four strains). Units I and IV shared the two subtype C strains. Male gender ($P < 0.02$), frequent hospitalizations ($P < 0.001$) and recent onset of hemodialysis treatment ($P < 0.05$) were positively associated with VRE colonization.
Conclusions: VRE colonization is reported for the first time among hemodialysis patients in Greece. Despite the low number of isolates, sharing of PFGE types in-between Units was demonstrated indicating spread of VRE clones among patients and, possibly, ineffective infection control measures.

P414 Effect of opening of a cohort unit on MRSA bacteremia rates

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Objectives: Following the opening of a MRSA cohort unit in January 2001, a significant reduction in MRSA acquisition rates within our hospital was seen. As MRSA colonization is a risk factor for MRSA bacteremia, it was postulated that this reduction in colonization may lead to a fall in MRSA bacteremia.
Methods: From January 2000 to June 2002, all episodes of *Staphylococcus aureus* bacteremia were recorded prospectively. MRSA bacteremia rates were compared before and after opening of the unit. Comparisons were also made with national data from EARSS.
Results: No significant reduction in MRSA bacteremia rates was seen following opening of the cohort unit. However, nationally, there was an increase in MRSA bacteremia during this period. Prior to the cohort unit, the MRSA bacteremia rate in our hospital closely correlated with the national average ($\chi^2 = 0.003$). After the unit opened our MRSA bacteremia rates were consistently lower than the national average, though not achieving significance ($\chi^2 = 2.26$).
Conclusions: Although MRSA bacteremia rates did not fall significantly after opening of a dedicated cohort unit, the rates remained similar and did not follow the increasing trend nationally. A cohort unit, by reducing MRSA acquisition rates in our hospital, may have contributed to the MRSA bacteremia rates remaining static and not mirroring the national increase during the study period.

P415 *Staphylococcus aureus* oxacillin-resistant: assessment of factors associated with acquisition in hospitalized patients

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Oxacillin-resistant *S. aureus* (MRSA) remain as an important pathogen detected in the majority of hospitals and is responsible for an increasing number of colonization and nosocomial infections. Recent reports have also noticed the emergency of community-acquired strains of MRSA. Many factors were associated with nosocomial acquisition of these resistant strains. Characteristics permitting recognition of patients harboring MRSA would aid infection control efforts to reduce intrahospital dissemination of such strains and choice of empiric therapy pending culture and susceptibility results. A prospective laboratory-based study was carried out with clinical isolates of *S. aureus* in a 550-beds tertiary care providing, university hospital, in Belo Horizonte, Brazil. Only one bacterial sample was selected for each patient. The strains were evaluated by using the presence of *mecA* gene, detected by PCR, as definitive criteria for MRSA and non-MRSA (MSSA). During four consecutive months, 103 patients were selected for interview and had their hospital records reviewed. Among them, 36 (35%) had MRSA and 67 (65%) had MSSA. Following the CDC criteria, 36 patients in MRSA group were classified as: three (2.9%) community-acquired infection, 26 (25.2%) nosocomial-acquired infection, seven (6.9) colonization; and 67 patients in the MSSA group as: 24 (23.3%) community-acquired infection, 32 (31%) nosocomial-acquired infection, 11 (10.7) colonization. Multivariate analysis identified receipt of antimicrobial agent in the 6 months preceding admission [odds ratio (OR) = 4.0; 95% CI, 1.3–12.8], presence of diabetes mellitus (OR = 4.9; 95% CI, 1.1–20.2); chronic break in the skin (OR = 3.2; 95% CI, 1.1–9.4); admission from other health care institution (OR = 4.0; 95% CI, 1.0–15.2); and >10 days hospitalization (OR = 3.4; 95% CI, 1.0–11.0) to be independently associated with the presence of MRSA strain. Further investigation of those three patients with MRSA, classified as having community-acquired infection, revealed that all of them had recent hospitalization and chronic peripheral ulcers, but could not fill the CDC criteria for nosocomial-acquired infection. Our date demonstrated that, in the population studied, some factors should be considered when making decisions about isolation and empiric antimicrobial therapy for patients with suspected staphylococcal infection. Community-acquired MRSA does not seem to be a problem for the moment.

P416 Central venous catheter-related infections: risk factors and the effect of glycopeptide antibiotics

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Objectives: Although central venous catheters (CVCs) have significant benefits in many clinical situations, the increase in their use over the last 20 years has been associated with at least a doubling of resultant nosocomial infections. We undertook a prospective study of all new central venous catheters inserted into patients in the intensive care unit, in order to identify the risk factors and to determine the effect of glycopeptide antibiotics on catheter-related infections.
Methods: All patients admitted to medical, neurosurgical and surgical ICUs of Istanbul Faculty of Medicine between January 2001 and December 2001 who submitted to CVCs were included in the study. The catheters used were triple lumen and made of polyurethane material (Arrow, Erding, Germany). The catheter was the first or any subsequent inserted into a patient. Current ward protocols were observed prior to catheter insertion. Catheters were cultured by semiquantitative method and blood cultures done when indicated. Data were obtained on patient age, gender, unit, primary diagnosis on admission, catheter insertion site, duration of catheterization, whether it was the first or a subsequent catheter and glycopeptide antibiotic usage.

Results: During the study period 300 patients with central venous catheters were prospectively studied. Ninety-one (30.3%) of the catheters were colonized and infection was found with 50 (16.7%) catheters. No significant differences were found for age, gender, unit, primary diagnosis, repeated catheterization and TPN use. Infection was diagnosed with higher rate in catheters inserted via jugular vein in comparison with subclavian vein (95% CI: 1.32–4.81, $P = 0.005$). The incidence of infection was higher in catheters which were kept in place for more than 7 days (95% CI: 1.05–3.87, $P = 0.03$). The incidence of infection was also higher in nonantibiotic using patients than patients who were using glycopeptide antibiotics during catheterization (95% CI: 1.49–5.51, $P = 0.005$). Of the 50 cases of CRI, most of the organisms causing infection were Gram-positive cocci ($n = 38$, 76%), with the most commonly isolated organism being *S. aureus* ($n = 32$, 64%).
Conclusion: Duration of catheterization and catheter insertion site were independent risk factors for catheter-related infection. Use of glycopeptide antibiotics during catheterization seems to have protective effect against catheter-related infection.

P417 Evaluation of catheter reinsertion as a risk factor for recurrent catheter-related blood stream infection (CR-BSI)

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Objective: To evaluate risk factors for recurrent CR-BSI following intravascular catheter removal.
Materials and methods: Patients who had CR-BSI followed by catheter removal and reinsertion between January 1998 to February 2002 at the University Hospital of Utah were included in the study. Recurrent CR-BSI was defined as positive blood cultures after three negative cultures, coupled with positive catheter tip culture or no other new source of infection evident.
Results: Twenty-five (28%) of 89 patients had recurrent CR-BSI; 16 patients with persistent bacteremia were excluded from the analysis presented here. Recurrence was higher among burn patients than other patient types ($P = 0.038$). Use of guide-wire exchange and time to reinsertion was similar across recurrent and nonrecurrent patient groups ($P > 0.5$). The first CR-BSI occurred a mean of 19 days after catheter insertion whereas recurrence developed a mean of 12 days after reinsertion. Coagulase-negative staphylococci were the most common cause of reinfection.

Characteristic	Recurrent infection	Non-recurrent	Total
No.	25	48	73
Age	45.8 ± 16.2	47.7 ± 16.1	
Gender, male	13	30	43
Diagnosis			
Burn	10	9	19
Malignancy	1	4	5
Transplant	0	5	5
Trauma	2	6	8
Type of catheter			
Multilumen	21	30	51
Hemodialysis	1	3	4
Peripherally inserted CVC	2	2	4
Hickman	2	7	9
Central Venous ports	0	2	2
Other	0	4	4
Organism			
Coagulase-negative staphylococcus	15	15	30
Staphylococcus aureus	6	17	23
Enterococcus	2	6	8
Pseudomonas aeruginosa	1	4	5
Other Gram-negative organisms	1	3	4
Candida species	0	3	3
Time intervals (day)			
Catheter insertion to CR-BSI	19.4 ± 42.5	20.9 ± 49.1	
CR-BSI to catheter removal	1.3 ± 1.8	1.8 ± 1.8	
Catheter removal to reinsertion	0.5 ± 1.5	0.2 ± 0.8	
Reinsertion to reinfection	12.1 ± 10.4		
Guide-wire exchange of catheter	8	12	20

Conclusion: Recurrent CR-BSI in this population was associated with admission to the burn service. Catheter reinsertion time was not a significant predictor of recurrent infection.

P418 Antibiotic lock therapy for the treatment of staphylococcal Hickman catheter-related bacteremia

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Pamplona, E

Objectives: Hickman central venous catheters are commonly used to provide vascular access in patients requiring prolonged intravenous treatment. Infection is one of the leading complications. Removal of the catheter is the mainstay of therapy in the management of these infections. The antibiotic lock therapy (ALT) has been suggested to be useful to treat catheter-related bloodstream infections in order to avoid catheter removal. This study carried out in a selected group of patients with Hickman catheter-related bacteremia (HCRB) due to staphylococci, analyzes the efficacy of ALT to treat these infections.
Methods: A total of 22 episodes of HCRB occurred in 16 patients. There was no tunnel tract or exit site infection in any of the cases. No evidence of other sources for the infection was assessed after a careful physical examination. To diagnose HCRB without removal of the suspected catheter, paired quantitative blood cultures (QBC) were obtained through the two lumens of the catheter and through a peripheral vein. Several solution locks were used: vancomycin (2 mg/mL) in 13 cases, teicoplanin (10 mg/mL) in 5, vancomycin (5 mg/mL) in three and teicoplanin (2 mg/mL) in one. Sodium heparin was added to these solutions. Antibiotic locks were instilled into the catheter lumen and were allowed to dwell for 12–72 h. The mean duration of ALT was 11.9 days (range 5–21 days). All the patients received a variable course of systemic antibiotics. QBC were obtained 72 h after the end of treatment. The persistence or recurrence of fever and bacteremia led to catheter removal and culture.
Results: Etiologies included coagulase negative staphylococci (20 isolates) and *S. aureus* (2). None of the catheters were removed within the antibiotic lock and no serious complications occurred during treatment in any case. Twelve episodes were successfully treated with one course of ALT (vancomycin was used in seven cases and teicoplanin in five). There was a relapse of the bacteremia in 10 cases (vancomycin was used in nine cases and teicoplanin in one); two of these catheters were removed within 72 h after the end of treatment and in the remaining eight episodes there was a response to a second course of treatment.
Conclusions: The majority of HCRB with staphylococci can be cured without device removal using ALT. Solutions locks containing teicoplanin seems more effective than vancomycin ones probably due to physical or chemical reactions of vancomycin with sodium heparin.

P419 Efficacy of antibiotic lock therapy to treat venous access port-related bacteremia

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Pamplona, E

Objectives: Venous access ports have become essential devices for the management of chronically ill patients. Port-related bacteremia (PRB) is a serious complication; in these cases the general approach is to remove the port. There are limited data concerning the efficacy of antibiotic treatment of PRB without port removal. We analyzed the outcome of 50 PRB treated with antibiotic lock therapy (ALT) plus systemic antibiotics.
Methods: Fifty episodes of PRB occurred in 45 patients (cases of *S. aureus* or yeast infections were excluded because ports were routinely removed to prevent more serious complications). There were no signs of pocket infection in any case. Paired quantitative blood cultures were obtained to diagnose PRB. Several solution locks were used: vancomycin (2 mg/mL) in 27 cases, teicoplanin (10 mg/mL) in 10, cefazolin (10 mg/mL) in three, vancomycin (2 mg/mL) plus gentamicin (2 mg/mL) in two, gentamicin (2 mg/mL) in one, levofloxacin (5 mg/mL) in one and trimethoprim-sulfamethoxazole (4/76 mg/mL) in one. Sodium heparin was added to these solutions except in the case of levofloxacin, in which macroscopic precipitation was noted. Antibiotic locks were instilled daily into the port and were allowed to dwell for 8–24 h. The mean duration of ALT was 11.68 days (range 3–21 days). All the patients received a variable cycle of systemic antibiotics according to the clinical course. Ports were removed when fever persisted beyond 3 days of therapy or in the event of persistent relapses.
Results: Etiologies included coagulase negative staphylococci (37 isolates), *Corynebacterium* spp. (5), *Propionibacterium acnes* (3), *Enterococcus faecium* (2), *Pseudomonas mendocina* (1), *Ralstonia pickettii* (1) and *Enterobacter cloacae* (1). No

serious complications occurred during ALT. Two ports were removed within 72 h after the end of treatment because of relapsing bacteremia. There was a response to ALT (defervescence and control negative blood cultures after the end of the treatment) in 44 (88%) episodes, and in the remaining four cases there was a relapse of the bacteremia. In all the relapses a second course of ALT was administered; infection was cured in one case and port was removed in three cases.
Conclusions: An attempt to treat PRB with ALT plus antibiotics, without port removal, is reasonable if the patient is clinically stable and has no signs of sepsis syndrome.

P420 Clinical features and management of cardiac device infections

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Objective: To review clinical and microbiological features of infected cardiac devices (CD) (pacemakers and automatic implantable defibrilator), diagnostic procedures and therapy and follow-up.
Methods: All medical records of patients admitted to Hospital de La Zarzuela with infected cardiac devices between January 98 and December 02 were reviewed in a protocolized manner. We considered a CD was infected if there was: (i) purulent drainage of generator pocket; (ii) a positive culture of the generator pocket; (iii) persistent bacteriemia without other obvious source in a patient with a CD with or without local inflammatory signs.
Results: Five CD infections (four pacemakers and one automatic implantable defibrilator) were diagnosed in the study period. All patients were male, with an age range between 53 and 70 years. They had no risk factors for infection, except one who was diabetic. Only two of the CD were implanted in our hospital and antibiotic prophylaxis was not administered. Previous manipulation was recorded in two cases, but no local complications preceding infection were described. Three patients had generator and cables infection and the remaining two had endocarditis. Blood cultures were positive in three of four cases. Echocardiogram was performed in every case with positive results in two cases. All but one had local inflammatory signs and fever was present in 80% of patients. The median time to diagnosis was 90 days. In one case *S. aureus* was responsible for the infection, in other patient the infection was caused by *Enterococcus* spp. and in two cases coagulase-negative staphylococci. Cultures were negative in the remaining patient. Treatment consisted of parenteral antibiotics and removal of the entire system except in one case which was treated only with antibiotics and debridation of generator. One out of four cases required extracorporeal surgery A pacemaker was reimplanted in all patients who required removal of the infected one. There were no relapses, with a median time of follow-up of 765 days.
Conclusion: CD infection is an uncommon but serious clinical problem. Early diagnosis is important in order to prevent systemic infection and endovascular dissemination; physicians should always consider this diagnosis in any patient with a permanent pacemaker or implantable defibrilator with fever or local signs. Aggressive management with antibiotics and removal of the system is usually curative. Reinfection of the new implants is unusual.

P421 Adsorption of antibiotics on functionalized polyurethanes as a strategy to prevent intravascular catheter-associated infections

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Objectives: Intravascular catheters are essential for the management of hospitalized patients, especially those admitted to intensive care units, even if microbial infections are frequently associated to their use. Microrganisms adhere to catheter surfaces by forming sessile multicellular communities encased in a hydrated matrix of polysaccharides and proteins, resulting in a slimy layer known as biofilm. The mode of microbial growth in biofilm is linked to the chronic nature of these infections and to their resistance to systemic antibiotic therapy.
Methods: The present research is focused on the development of in vitro experimental models to prevent catheter-related infections based on the adsorption of antibiotic molecules on polyurethanes, the most frequently employed polymers in catheter fabrication. The four antibiotics tested were cefamandole nafate, amoxicillin, rifampin and vancomycin. The amount of the antibiotics adsorbed was determined by UV-VIS spectroscopy while the kinetics of the antibiotic release from the polymers was studied performing washings in saline solution. The antibacterial activity of the realized polymer-antibiotic systems was assessed in vitro by the Kirby Bauer test and by the optical microscopy observation of the polymeric surfaces.
Results: To find out the chemical properties of the polymers optimizing both the antibiotic adsorption and release, we functionalized polyurethanes with acidic and basic groups. The presence in the polymer side chain of basic functional groups able to establish ionic interactions with the acidic groups of the tested antibiotic molecules resulted to be the better condition for antibiotic adsorption. As the antibiotic release is concerned, cefamandole nafate and vancomycin exhibited the strongest drug–surface interaction both showing a release value of about 15% in 48 h of washing vs. release values for rifampin and amoxicillin of about 30 and 40%, respectively. As the antibacterial activity is concerned the best results were obtained with cefamandole and rifampin adsorbed on basic polymers in that an inhibition zone was detected till 8 days and 8 months, respectively.
Conclusions: The satisfactory results obtained open new perspectives to realize intravascular devices recalcitrant to microbial colonization and providing long-term protection especially for patients hospitalized for severe diseases.

P422 Prospective evaluation of central line associated colonization or infection by five different methods

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Objective: To test five different methods to identify line sepsis in real time.
Methods: In a prospective, clinical-microbiological study all central lines (CL) inserted in our hospital over 6 months were included. Routine CL cultures were obtained daily; if positive, five methods for detecting bacteremia were applied: (i) coupled peripheral venipuncture-CL cultures with measurement of time until positivity, (ii) isolator counts, (iii) acridine orange stain, (iv) endoluminal brush, and (v) tip culture. Results were correlated with clinical status pertaining infection.
Results: The study included 78 catheters (35 femoral, 23 subclavian and 20 jugular) from 47 patients, and 321 blood cultures (of which 128, or 40% were positive). No specific point in time was found to mark a significant change in the rate of culture growth. There was a non-significant trend toward earlier contamination of femoral lines. A correlation was found between short time till growth in a blood culture and high colony counts in the isolator of the same sample. A small ratio of time to growth in the CL culture divided by time to growth in the peripheral culture correlated with line sepsis. A positive CL culture led to the drawing of 43 parallel CL-peripheral cultures, the results of which were classified as follows: (i) absence of growth of both central and peripheral cultures suggests transient colonization, not requiring treatment or line removal (14/43, 33%). (ii) Growth of the peripheral but not the CL culture indicates true sepsis, unrelated to the line, and the CL needs not to be removed (2/43, 5%). (iii) Growth of only the central culture after 24 h often indicates line colonization that may not require removal of the line, but observation only (8/43, 19%). (iv) Growth of the CL culture <24 h could suggest either line-associated sepsis or sepsis that is not line-related. Growth of the peripheral culture set at a significant time-interval after the central one became positive, may indicate line-associated sepsis, mandating removal of the line (8/43, 19%) or unrelated sepsis (11/43, 26%); however, these data do not allow for real-time separation and many physicians would remove a CL after a CL culture from a central-peripheral couple grew positive <12 h.
Conclusion: A positive blood sample, randomly drawn via a CL should lead to obtainment of a new CL culture, concurrent with at least one peripheral vein culture, with measurement of time until growth. Implementation of this approach could prevent unnecessary removal of as many as 24/43 (56%) of central lines.

P423 Molecular epidemiology of catheter-related sepsis caused by coagulase-negative staphylococci

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Objectives: Catheter-related sepsis (CRS) caused by coagulase-negative staphylococci (CNS) is common in patients with hematologic disease. It can be confirmed by isolation of bacteria of the same species and of the same type from peripheral blood and the catheter tip or port or from blood drawn through the catheter (CVC). Because clinical signs of CRS in these patients

are usually scarce and it is difficult to differentiate CNS sepsis from blood culture contamination, we tried to confirm by molecular typing that 14 patients with CNS isolated from peripheral blood and CVC had CRS.
Methods: In 14 patients CNS were isolated from peripheral blood, and in addition in nine of them from blood drawn through the catheter, in two from catheter port, and in three from catheter tip (CVC). Bacteria were identified and antimicrobial susceptibility to penicillin, oxacillin, erythromycin, clindamycin, rifampicin, gentamicin, ciprofloxacin, vancomycin and teicoplanin was determined according to NCCLS. Bacteria were typed by Pulsed Field Gel Electrophoresis (PFGE).
Results: In all 14 patients CNS isolated from peripheral blood and CVC belonged to the species *S. epidermidis*. In all patients isolates from blood were susceptible to vancomycin and teicoplanin, in one only to oxacillin, in three to erythromycin and clindamycin, in nine to rifampicin, in three to gentamicin, in two to ciprofloxacin and in none to penicillin. In 13 patients antibiotic susceptibilities from peripheral blood and CVC isolates matched, and in one they did not. PFGE patterns of *S. epidermidis* from peripheral blood and from CVC matched in 10 patients.
Conclusion: In 10 of 14 patients catheter-related sepsis was confirmed by identical PFGE patterns of *S. epidermidis* from blood and CVC. In four patients, where PFGE patterns were different, there was a possibility of mixed infection or of blood culture contamination.

P424 Colonization incidence of intravascular catheters and catheter-related bacteremia

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Objectives: The aim of this study was to evaluate the rate of catheter-related bacteremias (CRB) in relation to the incidence of intravascular catheters colonization (IVC).
Materials and methods: One hundred and forty eight intravascular tips of catheters were cultured in our laboratory in a period of 2 years (2000–2001) and also peripheral blood cultures from the same patients (pts). All specimens of catheters were processed with (i) the 'roll plate' semiquantitive method (Maki technique), (ii) qualitative after incubation in thioglycolate broth and subcultured in blood agar and McConkey agar. Blood cultures were incubated in automated BaCT/Alert System (Biomerieux). Identification to species level was performed by the automated Wider-Difco.
Results: Positive cultures were obtained from 48 (32%) of the 148 pts with (IVC) of which 30 pts (20%) had also positive blood cultures. Of the isolated 52 microorganisms from the catheter cultures coagulase-negative staphylococci accounted for 24 (46%), Gram-negative bacilli for 14 (27%), fungi for five (10%) and *Staphylococcus aureus* five (10%). Less often were isolated other microorganisms as *Corynebacterium* spp., *Enterococcus* spp., etc. (7%). *Acinetobacter* spp. were the most isolated microorganisms ($n = 9$, 17%) followed by *Pseudomonas* spp. ($n = 3$, 5%) and *Enterobacter* spp. ($n = 2$, 4%). In 12 cases (8%) the same pathogens were isolated from IVC and blood cultures. CNS represented 58%, fungi 25% and Gram-negative bacilli 17%.
Conclusions:
1. These results indicated that the colonization rate of IVC was (32%) according to the literature. The CRB rate was found to be 20%.
2. About the bacterial spectra in our patients the predominant pathogens was CNS followed by Gram-negative bacilli and fungi.
3. It is recommended that blood cultures should be combined with IVC-tips cultures because bloodstream infection could be caused by different bacteria than those of colonized IVC.
4. CRB was uncommon in our experience probably because of extended use of antibiotics and the right evaluation of the explantation time of the catheters.

P425 Biofilm forming capacity of neurosurgical *Staphylococcus epidermidis* isolates

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Staphylococcus epidermidis is a common cause of prosthetic device-related infections in the neurosurgical intensive care unit (NICU). Enzymes encoded by the staphylococcal ica operon synthesize a polysaccharide intercellular adhesin required for biofilm formation, which represents an important virulence determinant. The principle objective of this study was to conduct a genotypic and phenotypic analysis of biofilm forming capacity among NICU staphylococcal isolates. Nine *S. epidermidis* isolates were collected prospectively from CSF and ventricular drain tips in the NICU; five were associated with neurosurgical device-related infection and three were regarded as contaminants. All isolates were methicillin resistant. Using a PCR assay the majority of isolates (7/9) were found to contain the ica operon. Biofilm-forming capacity under standard laboratory and stress inducing conditions was evaluated in an attempt to more accurately reflect the role of the biofilm phenotype in vivo. Under standard laboratory conditions only one ica-positive isolate was capable of biofilm formation. However, under stress inducing conditions all seven ica-positive isolates were biofilm positive. The two isolates in which the ica operon was absent were unable to form biofilm under any growth condition. NaCl was the most effective biofilm-promoting stimulus and enhanced biofilm production in six isolates. Using RT-PCR, the role of ica operon expression in biofilm formation was demonstrated and revealed a strong correlation between ica operon activation when isolates were grown at 42°C and in NaCl, and enhanced biofilm formation. However, as individual clinical isolates had differential responses to the stress inducing conditions examined, the icaR regulatory gene and ica operon promoter sequence were sequenced to examine if genetic variation was responsible for the observed differential regulation of biofilm formation. However, no sequence variation was detected suggesting that a trans acting factor may contribute to differential regulation of biofilm formation in individual isolates. These results confirm that the presence of the ica operon alone is not sufficient for biofilm formation and that regulation of ica operon expression by local environmental conditions may play a key role in biofilm associated neurosurgical device-related infections.

Viral disease (not HIV, herpes or hepatitis)

P426 AFP surveillance in Kosova

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Purpose: Epidemiological characteristics of poliomyelitis situation in Kosova, the success of active AFP surveillance, possibilities for stopping circulation of polio virus by the year 2003 and eradication of the disease by the year 2005.
Methods: Surveillance data, epidemiological assessment by using descriptive component, the retrospective observation of the phenomenon by time, reporting data from active AFP surveillance, communicable disease surveillance and Immunization coverage reported in the Department of Epidemiology.
Results: Since 1991 through 2002, 54 suspected cases of poliomyelitis have been registered in the territory of Kosova with two lethal cases. The highest number of cases was noticed during 1996 (25), with morbidity of 1.16 and lethality of 4.0. Last 5 years no cases of poliomyelitis have been registered. Type I, II, III of 'wild virus' of polio was isolated in 12 cases. Nine cases were negative and other three were not typified. Last 4 years no case of poliomyelitis was recorded. During sub-NID in Kosova, which was implemented in six rounds in the year 1996 in the age-group 0–5 years with polio immunization were covered 97% of the children, in 1997 (93%) and 1998 (80%). During this period 80% of the children have been completely covered with immunization. Active surveillance of AFP has been undertaken from April 1997 through 2002, 38 AFP cases have been registered. During this period, the largest number of cases, 11 altogether, was registered in 1998, mostly from Prishtina region, where in total we have registered 14 AFP cases.
Conclusions: Thanks to high immunization coverage against poliomyelitis no cases of poliomyelitis have been registered within the last 5 years, it is foreseen that by the end of year 2003 the transmission of wild polio virus can be interrupted and the ground set for the eradication of the disease by the year 2005, the goal set up by health authorities in Kosova. To accomplish it, an active surveillance of polio has been undertaken as well as a vaccination target established for the next years. It is expected that the percentage of the

population to be vaccinated will be of 90% in 2002, 90% by 2003 and 95% in 2004.

P427 Rotaviral infections among child inpatients in a clinic of infectious diseases, Pilsen, Czech Republic. Results of a longitudinal study, 1986–2001

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Objectives: Rotaviruses are the most frequent etiological agent among patients with diarrheal diseases. Longitudinal data could be the basis for the start of vaccination.
Methods: The importance of rotaviruses was analyzed among children's inpatients during years 1986–2001. Detected rotaviruses from years 1999–2002 were serotypized with monoclonal antibodies.
Results: In years 1986–2001 were examined 4615 younger children with gastrointestinal diseases. Rotaviruses were observed among 25.6% patients, their occurrence changed in different years between 14 and 41%. The incidence of rotavirus acute gastroenteritis among different age groups was as follows: children under 7 months-15.0%, 7–12 months 28.2%, 13–24 months 32.0%, 25–36 months 27.7%, older 18.8%. The highest incidence rate was during March (43.9%), the lowest during November (16.5%). Among 1162 patients without gastrointestinal symptoms rotaviruses were detected 52× (4.5%). Nosocomial rotaviral infection was laboratory detected in 258 of 4410 infants repeatedly examined during the hospitalization (i.e. 5.8%).These infections protracted hospitalization on average by 4 days. G-serotypization was investigated in 260 stools. The type was detected 185× (i.e. 71.2%).The most frequent serotypes were types G1 and G3 (42, respectively 17%).
Conclusion: The results confirm frequent occurrence of rotaviral infections among hospitalized chidren. The distribution of serotypes is similar to other European countries.
Acknowledgement: Grant support: IGA MZ Czech Republic N. 6036–3.

P428 Prevalence of mumps virus antibodies in population of Poland

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Objective: Mumps is an acute, highly contagious viral disease. Although mostly causing a mild childhood disease, mumps virus may also affect adults, among whom complications such as meningitis and orchitis are relatively common. The annual incidence of mumps in Poland is in range of 100–570 per 100 000 population with epidemic peak every 2–3 years. Till now mumps vaccination has not been included in national immunization program in Poland. National decision to implement large-scale mumps vaccination should be based on sero-epidemiological analyzes. So, before establishing the mumps vaccination schedule the prevalence of mumps virus antibodies in population was carried out.
Methods: Anonymized residual serum samples were collected from individuals at age 1–30 years attending selected hospitals and Public Laboratories between February and October 2001. Two hundred and thirty samples were collected from each age group (1–4; 5–9; 10–14; 15–19; 20–24 and 25–30 years of age), giving a total of 1390 samples. Serum samples were assayed for mumps-specific IgG in human serum using a commercially available enzyme-linked immunosorbent assay kit (Virotech, Germany). Antibody concentrations were expressed in arbitral units per milliliter (VE/mL). Samples with titers below 9 VE/mL were regarded as negative.
Results: The annual incidence of mumps in Poland is in range of 100–570 per 100 000 population with epidemic peak every 2–3 years. The results of serological survey have indicated the differences in proportion of positive serum samples according to age. The proportion of positive serum samples (>11 VE/mL) was only 24.1% for children aged 1–4 years; 45.4% for children aged 5–9 years; 72.5% for age group 10–14 years and over 85% for persons aged 15–30 years. The age group 1–4 is most vulnerable for mumps infection, and this is a target group for vaccination.
Conclusion: The high proportion on individuals with negative titers of specific mumps IgG antibodies justifies the introduction of mass immunization against mumps in Poland. Because Poland has adopted a goal of elimination indigenous measles by the year 2007, the combination of mumps with measles and rubella vaccines instead of monovalent measles vaccine for children aged 13–15 months is recommended.

P429 Seroprevalence of *Varicella zoster* virus antibody in 2002 in Poland

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Objective: Varicella (chickenpox) is an acute, highly contagious viral disease with world-wide distribution. Due to its extremely contagious nature, varicella is experienced by almost every child or young adult. During 1990–93 VZV, infections in Poland about 220 000 cases were reported; during last 3 years this number decreased and oscillates about 100 000. Of these, approximately 759–1200 required hospitalization and 2–3 died. VZV vaccine is not foreseen in current Vaccination Program in Poland. Surveillance for VZV infection and seroepidemiological studies will be necessary for evaluation of vaccination efficacy and timing of booster dose. Surveillance of Herpes Zoster will bring more precise information on extent of VZV infection
Methods: Seroprevalence study on VZV antibodies in Poland comprised 1048 persons, up to 40 years old. Among them were 559 females and 489 males. Anonymous serum samples collected from venous blood were obtained from three hospitals in Warsaw and six other places. For serological testing commercial test kit was used, Enzyme Immunoassay for the determination of IgG antibodies to VZV in human serum or plasma′, manufactured by Hycor (Germany), distributed by Dia Sorin (Italy).
Results: Results of our study are following. The examined sera with anti VZV antibodies in relation to patients' age Percentage of positive results was highest in age group 15 and remained on similar, constant level about 70–80% in older age groups. Children up to age 15 are weakly protected from VZV infection. Age group 0–5 is most vulnerable for infection-protective level of anti-VZV antibodies had maximum 10% of children and this is the target group for vaccination. In older age groups seroconversion rate grows, but still only 40–60% of children age 10 had antibodies. Our study displayed no statistically significant differences within age groups between females and males. Distribution of anti-VZV antibodies among females is as follows: 73% of results were positive and 27% were negative.
Conclusion: Results of our seroepidemiological studies and experience of countries vaccinating against VZV indicates that mass vaccination in Poland would modify epidemiological situation by reduction of chickenpox cases indicate for necessity of vaccination in Poland.

P430 Link between prevalence of human Papillomavirus D. infection in the upper respiratory tract of mother and her child

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Objectives: Our aim was to evaluate the link between prevalence of human papillomavirus (HPV) infection in the upper respiratory tract of mothers and their children.
Methods: The group of random selected 110 children (47 girls and 63 boys, mean age, 2.7 years, range, 1–8 years) were treated for upper respiratory tract infections in the Kaunas Clinical Hospital of Infectious Diseases, and these children's mothers (mean age, 27.2 years, range, 19–43 years) were examined. Routine laryngological examination was performed. Pharyngeal swabs of all persons were taken and analyzed for the presence of HPV DNA. Polymerase chain reaction (PCR) was performed as described by Tucker *et al.*
Results: HPV infection was detected in 48.18% (53/110) of children. HPV DNA was determinate in nearly one third (35/110, 31.82%) of mothers. HPV infection was established in children of HPV positive mothers in almost all cases (94.29%, $P < 0.001$). Mostly viral types identified in HPV positive mothers coincided with viral types identified in their children (in 57.58% of cases). In 24.24% of cases viral types were only partly coincident. There were identified only low-risk HPV types 6 and 11 in children and low-risk HPV types 6 and 11 with high-risk HPV type 16 in their mothers in all partly coincident cases. There were identified different HPV types in 18.18% of cases of HPV positive mothers and their children. There were identified only high-risk HPV-16 in mothers and only low-risk HPVs (type 6 and 11) in children in all above-mentioned conflicting cases of detected viral types. In

order to make certain that HPV infection of the upper respiratory tract can be prognosed for child if his mother has HPVs in her upper respiratory tract, a correlation test was carried out. It was established that odds ration of HPV infection of the upper respiratory tract for child increases 45.3 times, when his mother has HPVs in her upper respiratory tract. Probability, that child will have HPV when his mother has HPV, is 0.94.
Conclusions: HPV persistence in the upper respiratory tract of mother is a significant important factor, allowed to make the prognosis of HPV infection of the upper respiratory tract for her child.

P431 A survey of Crimean-Congo hemorrhagic fever in Iran

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Objectives: Crimean-Congo Hemorrhagic Fever (CCHF) is a zoonotic arboviral disease caused by a virus of Nairovirus genus (Bunyaviridae) and is transmitted by tick bite and nosocomially. After an epidemic of Viral Hemorrhagic Fever (VHF) in 2000 in Iran in a survey from 2000 to 2002 by the lab of arbovirus of the Pasteur institue of Iran with collaboration of the WHO reference center (Pasteur institue of Dakar), we tested 450 suspected patient sera for CCHF and some other VHFs like Yellow Fever (YF), Rift Valley Fever (RVF) and Dengue2 (D2).
Methods: We have tested the sera by specific ELISA method for IgM and IgG detection against CCHF, YF, RVF, and D2 and also by RT-PCR technique for detection a fragment in the small segment of the CCHF virus genome.
Results: CCHF results, the results show between 450 suspected cases, there is 178 IgM positive cases (39.55%), 166 IgG positive (36.8%) and 31 RT-PCR positive cases most of the IgM positive cases (confirmed cases) are from the south-east of Iran (neighbourhood of Afghanistan).
The numbers of suspected and confirmed cases according to the years are as follow: 2000:56 suspected, 20 confirmed, 4 deaths; 2001:166 suspected, 61 confirmed, 7 deaths; 2002: 228 suspected, 97 confirmed, 7deaths; 70% of patients were male and 30% female
All confirmed cases had the three main clinical signs of CCHF disease, like: fever hemorrhages and thrombocytopenia. Between confirmed cases 35% were farmers and butchers. Note: All sera were negative for YF, D2 and RVF.
Conclusion: Our results show CCHF is the main etiological cause of VHFs in Iran. Because the viremia period is very short in CCHF patients we found a few RT-PCR positive samples, therefore analysis of the humoral response (IgM and IgG) is the best way of detection. The increase in the number of patients in these 3 years, could be due to a rising incidence of the disease in Iran, or as a result of improved case finding by physicians and also due to progress in the diagnostic methods in our lab.

P432 Outbreak of measles in Kaunas, Lithuania

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The aim of the study: to investigate the epidemiological and clinical data of measles infection in adults during the measles outbreak in Kaunas in year 2002.
Materials and methods: Prospective analysis of 34 measles cases in adults, treated at Kaunas hospital of infectious diseases from February through May, 2002. Diagnosis of measles was confirmed by detection of IgM antibodies against measles virus in serum.
Results: An outbreak started with a cluster of four cases during the second decade of February. The index case traveled to Poland within 3 weeks before the onset of disease. The median age of measles cases was 25 years (range, 17–36 years). Twenty-five (74%) of patients were females. Two out of 34 (6%) patients had received a single dose of vaccine against measles, in 32/34 (94%) patients the vaccination history was not known. In four cases nosocomial transmission of measles to hospital staff has been established. Complications of measles were observed in 14/34 (41%) of cases. Most common complication of measles was pneumonia in 8/34 (24%) cases. Otitis media and tonsillitis were diagnosed for 2/34 (6%) cases each, meningoencephalitis and sinusitis for 1/34 (3%) cases each, respectively. No mortality due to measles was observed during the study period. In all but one case diagnosis of measles was confirmed by detection of IgM antibodies against measles virus in serum. In one case diagnosis was based by typical clinical picture and contact within 3 weeks with a person with laboratory confirmed infection.
Conclusion: Measles in adults is important infection due to high rate of complications. Efforts should be made towards extension of vaccination against measles for young adults.

P433 Hepatitis in hemorrhagic fever with renal syndrome

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Background: Bosnia and Herzegovina is endemic area for hemorrhagic fever with renal syndroma (HFRS) caused with virus from Hantaan group. The Hantaviruses are viral species in the Bunyaviridae family of viruses. Until now few species as Fojnica I, II and III and Sarajevo, have been isolated in our patients.
Objective: To point out to hepatotropic potential of Hantaan virus.
Methods: During 2002, 20 hospitalized patients due to hemorrhagic fever with renal syndrome (HFRS) were enrolled. Diagnosis was etiologically confirmed by enzyme-linked immunosorbent assay (ELISA), with detection of antihantaviral-specific immunoglobulin M (IgM). Three patients out of 20 came from urban centers and rest of them (17) were from rural areas. The patients were ranged in age from 10 to 63 years with average age of 31; four were female and 16 were male. The patients with previous hepatic diseases were excluded at the beginning.
Results: On admission to the hospital, 18 patients out of 20 had elevated concentrations of liver transaminases. At the beginning the values of these enzymes were modest. During the progression these values were growing with no correlation with level of azotemia. The levels of liver transaminases declined to average values, till the end of first month. Modest elevation of bilirubin was found in just three patients. The enlargement of liver observed by ultrasonography, were found in total number of patients. One patient died due to hemorrhagic shock caused by excessive fibrinolysis in terminal phase of renal insufficiency. For other patients final outcome was complete recovery.
Conclusion: Since 18 out of 20 patients had verified hepatitis, Hantaan virus with its possible hepatotropic effect can complicate in addition clinical picture and prognosis of this severe type of hemorrhagic fever.

P434 Unusual presentation of an acute common Epstein-Barr virus infection

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The spectrum of infections associated with Epstein-Barr Virus (EBV) ranges from self-limited mononucleosis in normal hosts to progressive infections in patients with disorders of immunity. We describe the case of a 2 1/2-year-old-boy with a 4-day history of high fever, maculo-papular rash with petechiae and upper respiratory tract infection. Twenty-four hours before admission, he developed an important edema of the lower limbs. On admission, the child was pale with generalized edema and florid petechial cutaneous rash including palms and soles. At physical examination, generalized lymphadenopathy and firm hepatosplenomegaly were also noted. Blood analysis showed marked pancytopenia, high C-reactive protein, low serum albumin and biological signs of dehydration. EBV specific IgM were increased in the absence of IgG. EBV blood antigenemia was very high (100.000 copies/mL). Lymphocyte population and immunoglobulin levels were normal. Initially, the severity of this EBV disease suggested the diagnosis of hemophagocytic lymphohistiocytosis; prolonged high fever, pancytopenia and hypertriglyceridemia were present but myelogram was normal and didn't demonstrate any sign of erythrophagocytosis. Clinical course was marked by an increasing hepatomegaly (reaching 15 cm below the costal margin), without any liver failure. Evolution was otherwise spontaneously rapidly favorable. At discharge, pancytopenia had resolved and EBV antigenemia had fallen to

35 000 copies/mL. This child presented an unusual dramatic form of acute EBV infection in the absence of any predisposing factor.

P435 Detection of Epstein-Barr virus genes in aggressive non-Hodgkin's lymphoma using polymerase chain reaction

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Epstein-Barr virus (EBV) has been found as an asymptomatic infection in all human communities. It is associated with many lymphoid neoplasms. In vitro studies showed that activation of EBV latent genes protect human lymphocytes from apoptotic cell death. The frequent association of EBV sequences with many lymphoid malignancies raises the hypothesis that EBV has a direct role in lymphomagenesis. Egypt is a North African country that differs markedly from equatorial Africa both ethnically and environmentally. The aim of the present work was to determine the frequency of EBV infection in NHLs in Egypt and whether viral infection contributed to clonal expansion and malignant transformation or not. Also to study the frequency of EBV subtypes in order to identify the pattern of Egyptian NHL whether endemic or sporadic. As well as to investigate the correlation of bcl-2 expression with that of the latent EBV gene. Forty retrospective cases of Non-Hodgkin's Lymphoma (NHL) were classified as follows: 15 cases large cell lymphoma, 15 cases Burkitt's lymphoma (BL), 10 cases lympholastic lymphoma. Ten cases of reactive follicular hyperplasia (non neoplastic) as control. All cases were analyzed by PCR assay for the presence or absence of multiple EBV genotypes (EBNA-3C), and clones (LYDMA gene). Bcl-2 immunohistochemical study was performed to clarify the correlation of bcl-2 expression with the latent EBV gene. Our findings revealed hat EBV was detected in 34 cases of aggressive NHLs, all cases were found to be polyclonal by LYDMM gene, PCR assay was of type 2 EBV. For control cases, all were positive for polyclonal EBV type 2. Large cell lymphoma, BL and lymphoblastic lymphoma were 80%, 87% and 90% positive for EBV, respectively. As regards the immunohistochemical findings it was found that 67.5% were positive by immunoistaining for bcl-2 while 32.5% were negative. From the positive cases 87% were large cell lymphoma, 56% were BL, and 50% were lymphoblastic lymphoma. All the control cases were negative for bcl-2. It is concluded from the present study that a significant correlation exists between the expression of bcl-2 and HBV infection as well as the different histologic grades of NHLs. It seems that the tumorogenic role of EBV is doubtful. It does not precede to development of tumor, possibly it has occurred after clonal expansion.

P436 Detection of human Papillomavirus and Epstein-Barr virus in tumors of head and neck region using polymerase chain reaction

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Fifty cases of head and neck (NH) tumors were included in this study. Biopsies included 10 nasopharyngeal carcinoma (NPC), two laryngeal polyps, nine squamous cell carcinomas (SCCs) of the larynx, one adenocarcinoma of paransal sinuses (sinonasal carcinoma), four carcinomas of salivary glands, one anaplastic carcinoma of thyroid gland, three SCCs of the lip, four SCCs of the tongue, two fibroepithelial polyps of the tongue, four facial skin carcinomas and 10 metastatic SCCs of cervical lymph nodes (LN) (of unknown primary). PCR assay was used to detect Epstein-Barr Virus (EBV) nuclear antigen 1 (EBNA-1) gene to detect the presence of EBV. In order to determine the clonality of EBV in the EBNA-1 positive cases, primers specific for LYDMA gene were used. Primers directed against the EBNA-3C gene were used to differentiate between type 1 and type 2 EBV strains. PCR using general primers targeting the HBV L1 region and HBV 16 E6 A1 B/E6 A2 specific for the E6 open reading frames were used for screening human papilloma virus (HPV) DNA and detecting HPV 16, respectively. Genomic EBV EBNA-1 was detected by PCR in 30 of 50 (60%) head and neck (HN) tumors. Using LYDMA gene PCR assay, 12 of the previous positive tumors (40%) were monoclonal indicating that EBV was the rate-limiting step in carcinogenesis. All of NPCs were positive for EBV EBNA-1 and were all monoclonal. In addition one of nine cancer larynx and one of the cancer lip were proved to be monoclonal. For the human papillomavirus (HPV), 8 (16%) of 50 HN tumors were positive using HPV genral primers in assay (GPs). Seven (87.5%) of those positive tumors were PCR positive for the highly oncogenic HPV 16 DNA. Positive tumors were NPC (10%), SCC of larynx (22.5%), sinonasal carcinoma (100%) SCC of lip (33.3%), SCC of tongue (25%) and one (10%) metastatic SCC of cervical LN. Coexistence of EBV-DNA (monoclonal for LYDMA gene) and HPV-type 16-DNA was observed in two tumors in our study (one was nasopharyngeal carcinoma and the other was SCC of lip). Results of our study concluded that EBV (type 2 strain) and HPV-16 play an important role in carcinogenesis and are associated with a far greater spectrum of HN tumors than expected. PCR is recommended as a useful and reliable method for detection of EBV and HPV-DNAs within the tumor cells. Also our results indicate that when performing PCR assay for HPV-DNA in HN tumors, it is enough to use only the set of GPs.

P437 Experimental oral coxsakievirus B4 infection of mice

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Objectives: Mice infected intraperitoneally have been widely used to study the pathogenesis of coxsackieviruses (CV). Since under natural conditions enteroviruses infect their hosts via the gastrointestinal tract, the interaction of the virus with the gut-associated lymphoid tissue may influence the course of infection.
Methods: The viral kinetics and histopathological changes were followed in different organs after the oral gavage of immunocompetent Swiss albino mice (strain ICR) with CVB4. For location of viral antigen in the small intestine, immunohistochemistry was employed.
Results: In the acute stage of infection the virus replication was observed in the heart, spleen, thymus, pancreas, small and large intestines. Infection was accompanied by viremia and by histological evidence of myocarditis but absence of the changes in the exocrine and endocrine pancreas. A longitudinal presence of the infectious virus was shown in the spleen and in the small intestines (till day 52 p.i.). The viral antigen was located in the smooth muscles of the muscular wall of the small intestine by immunohistochemistry. Though mild myocarditits was observed as early as on day 10 p.i., necrotic changes in the myocard with mononuclear infiltration and fibrosis were demonstrated on day 71 p.i.
Conclusions: The oral route of infection provides an excellent model for studying the pathogenesis of CVB infections.

P438 The efficacy of oral Ribavirin in the treatment of 81 proved cases of Crimean-Congo hemorrhagic fever in Iran

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Crimean-Congo Hemorrhagic fever (CCHF) is a lethal hemorrhagic fever caused by a tick-borne virus. There are few reports on the efficacy of oral ribavirin in the treatment of CCHF patients. This study was designed as a historical cohort with 187 clinically suspected cases since June 1999 to the end of September 2001. Only 139 of 187 suspected cases were treated with oral ribavirin based on the availability of the drug. Eighty-one of 187 cases were serologically confirmed to have the disease. The two groups (treated and nontreated) were compared for the incidence of outcome (Survival). Ninety-seven (69.8%) of 139 suspected treated cases and 61 (88.4%) of 69 confirmed treated cases were survived. Based on this study, the efficacy of oral ribavirin is measured equal to 80 and 34% in confirmed and suspected groups, respectively. We conclude that oral ribavirin is an effective treatment for hemorrhagic form of CCHF patients. We should be aware of the limitations of observational studies. However, there is no Randomized Control trial in the literature, and it can not be performed in future due to medical ethics. Therefore the results of this study could provide a valuable information in deciding how to treat CHF patients.

Clinical and epidemiological aspects of hepatitis (not HCV)

P439 Evaluation of a new automated assay for the detection of HBs antigen on the VIDAS analyzer

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Objective: The detection of HBsAg is used as the first-line diagnostic test for hepatitis B virus infection. It is also used for the follow-up of chronic carriers. VIDAS HBsAg Ultra is a new assay for the qualitative detection of hepatitis B surface antigen on the VIDAS analyzer using the ELFA technique. This study was performed to determine the sensitivity and specificity of this new assay.
Methods: Fresh samples were tested in parallel with the VIDAS HBsAg Ultra and ABBOTT AxSym HBsAg (V2) assays during a multicenter study performed in France in 2002. The VIDAS HBsAg Ultra assay is easy to use and can be run in 60 or 90 min (short and long protocol). The SPR, which is the solid phase receptacle is coated with two monoclonal anti-HBs antibodies selected for their binding capacity towards wild type as well as modified HBs antigen. After incubation and washing steps, the sandwich complexes are detected by the substrate (4-methyl-umbelliferyl phosphate), giving a fluorescence signal proportional to the concentration of antigen present in the sample. The tested population was determined according to the common technical specificities requested for the CE marked registration. For the sensitivity determination, the population combined 400 positive specimens including subtype consideration and 20 seroconversion panels. The analytical sensitivity was assessed for the VIDAS HBsAg Ultra assay using the French standard (ng/mL), as well as the two standards from the National Institute for Biological Standards and Control (IU/mL). For the specificity determination, 5000 unselected donors, 200 hospitalized patients and 100 potentially cross-reacting blood-specimens were tested.
Results: The analytical sensitivity was found to be 0.12 ng/mL and 0.08 ng/mL using the short and the long protocol, respectively. The specificity was determined as being 100%. This study provides data comparing the clinical specificity and sensitivity of the VIDAS HBsAg Ultra assay vs. the ABBOTT AxSym HBsAg assay by using seroconversion's panels, panels from the Etablissement Français du Sang and also serum samples from patients with chronic and acute hepatitis B.
Conclusion: These results demonstrate that the VIDAS HBsAg Ultra assay combines a high sensitivity and specificity with the advantages of a rapid and reliable fully automated immunoanalyzer.

P440 Determination of hepatitis B virus (HBV) and genotype D with simple PCR

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Objectives: Different HBV genotypes have shown characteristic geographical distributions, which is important epidemiologically. HBV strains have been classified into eight different genotypes, of which genotype D is prevalent in Turkish population.
Methods: HBV genotype D shows a 33-bp deletion in pre-S1 region that explains their smaller genomic size (3182 bp). This deletion facilitates the identification of genotype D. For discrimination of genotype D from other genotypes with PCR a pair of primers in pre-S1 region was designed. Genotype D easily could be differentiated from other genotypes with using size of PCR products such as 79 and 112 bp in with genotype D and A, respectively, in agarose gel electrophoresis.
Results: Forty-four sera which were identified as genotype D ($n = 40$) and genotype A ($n = 4$) with restriction fragment length polymorphism (RFLP) method were included in this study. With using this PCR method, the genotypes were correctly identified and also it this test was able to detect HBV DNA at 1000 genomes/mL.
Conclusions: In conclusion, this method is quick (~5 h) and it will contribute to epidemiological study of HBV in high-prevalence area of genotype D also it simultaneously can determine genotype D and HBV DNA positivity.

P441 Prevalence of hepatitis B infection in Bandar Abbas, Iran

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Objectives: Early diagnosis of sexually transmitted diseases such as Hepatitis B is critical for maternal and infant health. The objective of this study was to estimate the prevalence and the risk factors of hepatitis B virus (HBV) infection in pregnant women in Bandar Abbas, south of Iran
Methods: A cluster sampling method with a sample size 540 pregnant women attending antenatal clinics at the university hospital of Bandar Abbas between March 2001 and February 2002 was used. Sera from all samples were assayed for hepatitis B surface antigen (HBsAg) by a commercial enzyme linked immunosorbent assay kit. A questionnaire, including demographic details of all sample units such as age, occupation, jaundice or hepatitis history, remarriage history, transfusion history, parity, history of treated for sexually transmitted diseases (STD) was completed for each sample unit.
Results: Prevalence of hepatitis B virus in pregnant women was estimated as 2.2% (95% CI: 1–3.5%). One infected case with jaundice history and another case with hepatitis B history was found. The results revealed an inverse relationship between the infection and education level (relative risk for low level education = 2.21; 95% CI: 1.3–5.4; $P < 0.05$) but no statistical association was found between hepatitis B infection and age, parity, history of previous treated STDs, anemia, blood transfusion, jaundice history and remarriage history ($P > 0.05$).
Conclusion: Based on these preliminary results, HBsAg test as a routine test for hepatitis B infection in the area's population is recommended.

P442 Two-year study of hepatitis B infection prevalence in a major Greek hospital

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Objectives: The purpose of this study was to perform a serological analysis of hepatitis B virus (HBV) infection prevalence in the patients of a Greek major hospital within a 2-year period.
Methods: During the two last years 2001–2002, sera from 12 632 patients (10 472 in-patients and 2160 out-patients), were tested for the detection of HBV markers. There were two groups of patients, Group A: 4052 patients (2996 in-patients, 1056 out-patients) who were controlled for HBV surface antigen (HBsAg) and Group B: 8580 patients (7476 in-patients and 1104 out-patients) who were controlled for the HBV markers: HBsAg, HBeAg, anti-HBe, anti-HBcore, anti-HBs. All tests were performed by MEIA methodology (AXSYM-ABBOTT).
Results: In Group A of the 2996 in-patients and 1056 out-patients were found to be positive for HBsAg 60 (2%) and 33 (3.1%), respectively. In Group B of the 7476 in-patients and 1104 out-patients were, respectively: 3488 (46.7%)−488 (44.2%) seronegative for HBV markers, 488 (6.5%)−138 (12.5%) positive for HBsAg, 1184 (15.8%)−69 (6.3%) positive for anti-HBs due to HBV vaccination, 2316 (31%)−409 (37%) negative for HBsAg and positive for the rest HBV markers. Of the 488 in-patients and 138 out-patients with positive HBsAg were found to be positive, respectively, for HBV markers: 392 (80%)−123 (89%) for HBsAg–anti-Hbcore–anti-HBe, 40 (8.2%)−15 (11%) for HBsAg–anti-HBcore. Of the 2316 in-patients and 409 out-patients with negative HBsAg and positive the rest HBV markers were, respectively, positive: 880 (38%)−139 (34%) for anti-HBs–anti-HBe–anti-HBcore, 888 (38.3%)−99 (24.2%) for anti-HBs–anti-HBcore, 340 (14.7%)−75 (18.4%)for anti-HBcore, 208 (9%)−96 (23.4%) for anti-HBcore–anti-HBe.
Conclusions: This study showed that there is a significant prevalence of HBV infection in the patients of our hospital (37.5% for in-patients, 49.5% for out-patients). The effort for the prevention must be continual and the control measures with the systematic hepatitis B vaccination should be strictly enforced.

P443 A review of hepatitis B transmission in an organized registry center, Tehran, Iran

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Objectives: Hepatitis B viral (HBV) infection is a worldwide disease. HBV is mainly parentally transmitted via blood and blood products or by sexual or prenatal exposure. In this study, we examined the routes of HBV transmission among the patients who were referred to our center.
Methods: A case-series study was conducted in patients who were seropositive for hepatitis B surface antigen (HbsAg) in Taleghani Hospital, Tehran, Iran, during July 2001 to July 2002.
Results: A total 260 patients (173 males, 87 females) were studied with mean age of 39.9 ± 14.9 years and a range of 9–98 years. Possible routes for HBV transmission were: family history of HBV infection (23.4%), history of major surgery (15.3%), history of transfusion (13.4%), shared shaving razors (10.4%), cupping (8.8%), tattooing (8.4%), history of HBV in mother (6.5%), history of HBV in spouse (4.2%), suspicious sexual contact (3.8%), intravenous drug abuser (2.6%) and the history of dialysis (0.3%). A total of 35.7% of cases seemed to have no risk factor.
Conclusion: According to our results, HBV transmission in Iran is similar to other countries with moderate prevalence. In our study, many of the patients did not express any risk factor. So, the education can help to prevent spreading of the virus.

P444 Prevalence of HBsAg in Albanian blood donors

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Background: In order to complete the national epidemiological data collected each year in Albanian blood donor population, a national prospective study has been undertaken since 2000 to determine the prevalence of HBsAg in blood donors.
Material and method: In this study are tested 18 876 donors. From them 9.4% (1781 donors) have been voluntary donors (VDs), 31.2% (5890) family replacement donors (FDs), 58.2% (10 990) regular donors (RDs) and 1.13% (215) first time donors (FTDs). The sample are tested in NBTS for HBsAg by ELISA method (ABBOTT third generation). The data are collected from individual sheet. Chi-square and *Z*-tests are used for statistical analysis.
The result: The prevalence of HBsAg in blood donors is 3.4%. In voluntary donors the HBsAg prevalence is 7.5‰, FDs 8%, RDs 0.2‰ and FTDs 9.8‰. Over the 3-year periods in voluntary donors the HBsAg prevalence ranged from 4.5 to 8.5%, in family replacement donors from 7.9 to 8.2%, in regular donors from 0 to 0.4% and first time donors from 3.2 to 14.2%. We observed that the HBsAg prevalence has a decline tendency both in VDs (8.5–4.5%) and RDs (0.4–0%) and a increasing tendency in FDs (7.9–8.2%) and FTDs (3.2–14.2%). According to sex the HBsAg prevalence in men is 9.4% and women 3.8%. Over the 3-year periods the prevalence in men has a decline tendency (9.8–8.5%) and increasing tendency in women (2.5–5.2%). According to age group, the HBsAg is 10.5% in 30–39 age group, 8.8% in 17–29 age group, 5.8% in 40–49 age group, 3.5% in 50–60 age group.
Conclusion: The prevalence of anti-HCV is higher in FTDs (9.8%) and FDs (8%) than VDs (7.5%) and RDs (0.4%). According to sex the prevalence is higher in men than women. According to age group the prevalence is higher in 30–39 age group. The higher HBsAg prevalence in blood donors explain with higher prevalence in general population. The decreasing HBsAg in blood donors explain with improved blood donors screening and selection method, and vaccinated of general population for HbsAg since in childhood. Considering the quality of blood we always recommend for voluntary blood donations.

P445 Nosocomial acute viral hepatitis in a teaching hospital of infectious diseases, Romania – results of a 4-year study (1998–2001) after introducing CDC case definitions

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Objectives: To establish the impact of presumed nosocomial acute viral hepatitis type B, C and non A–C (AVH) in the Cluj-Napoca Teaching Hospital of Infectious Diseases, after introducing the hepatitis B vaccination in the National Vaccination Programme (1995) and the confirmatory serological testing for acute viral hepatitis A, B and C (1998).
Methods: We retrospectively studied all AVH (1916 cases) admitted in the Cluj-Napoca Hospital of Infectious Diseases between 1998 and 2001. We designed a database using the medical records comprising of: demographic data, premorbid conditions, questionnaire about possible nosocomial and other exposures, bilirubin, SGPT, other biochemical tests, the main serological markers for AVH (IgM anti-HAV, IgM anti-HBc, third generation HCV EIA). EPI6 software was used for statistical analysis.
Results: The incidence rates of AVH type B were significantly decreasing representing 254 cases −13% of all cases, while type C and non A–C AVH demonstrated an increasing trend with 201 cases – 10.4% of all cases. Presumed nosocomial hepatitis type B and C represented 30 and 34.8%, respectively. Major perceivable risks for nosocomial AVH type B were surgical and dental procedures (32 and 29.7%, respectively), with only one documented post-transfusion AVH. The same major risks were established for AVH type C (48 and 22%) but with a much higher risk for postransfusional AVH, eight cases, 11%. AVH type B and C in dialyzed patients occurred in 5.3 and 4.3% of presumed nosocomial cases. No significant differences in clinical manifestations and immediate prognosis were found in comparison with the community-acquired AVH type B and C.
Conclusions: The WHO eradication project of hepatitis B demonstrates very good results with only three cases in children, under a good notification of cases (mandatory hospitalization for all AVH). Nosocomial exposures might be less important than revealed due to an increased perception upon the hazards represented by medical procedures, still they should be considered. Post-transfusional AVH type C remains higher than expected probably due to paid blood donations.

P446 Fibronectin levels in chronic viral hepatitis and response of this protein to interferon therapy

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Objectives: In this study, the plasma fibronectin levels in the cases of chronic hepatitis B and C infection and this protein's response to the interferon therapy were examined.
Methods: Totally, 38 patients of chronic hepatitis, 21 of them being hepatitis B, 17 of them being hepatitis C; and 24 healthy blood donors, as the control group, took part in this study. The quantitative determinations of fibronectin in plasma samples were performed with the Bohring Nephelometer BN 100 (N Antiserum to Human Fibronectin, code no OUND, Dade Behring Marburg GmbH, Marburg Germany).
Results: It was observed that the fibronectin plasma levels of the control group were significantly higher than those of the patient group before the therapy ($P = 0.043$). After the interferon therapy of 6 months, the difference between the fibronectin levels of 16 examined patients before and after the treatment was found significant ($P = 0.001$). A negative correlation was detected between the fibronectin levels before the therapy and the inflammatory grade as far as the histopathology of the illness was concerned ($r = -0.49$), which is a statistically significant value ($P = 0.002$). The correlation between the levels of fibronectin and the stage of the fibrosis was found insignificant statistically ($P = 0.225$). When comparing the levels before and after the therapy, as far as ALT and AST values were concerned, it was observed that both parameters fell significantly after the therapy ($P = 0.002$). However, no correlation was observed between the fibronectin levels and ALT, AST before and after the therapy. To conclude, fibronectin can be a useful marker for showing the hepatic inflammation and damage in the cases of chronic hepatitis, and can also be used in the evaluation of the response to the interferon therapy like other bio-chemical parameters (ALT, protrombin activity, etc.).

P447 Neuropeptidergic control of immune response, following antiviral treatment, in chronic hepatitis B patients

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Objectives: Lamivudine is a nucleoside analogue with potent antiviral activity against hepatitis B virus (HBV). Pituitary adenylate cyclase activating polypeptide (PACAP) is a multifunctional neuropeptide, produced within the

lymphoid microenvironment, inducing the production of Th2-type cytokines. The aim of our study was to investigate the possible alterations of plasma PACAP-38 levels, in chronic hepatitis B (CHB) patients, during lamivudine treatment and to compare them with the biochemical, virological and histological data.
Methods: Plasma PACAP-38 levels were measured in 25 CHB patients, before the beginning and after the completion of a 52-week lamivudine treatment period and in 22 healthy blood donors (HD), using competitive radio-immune analysis (RIA). Statistical evaluation of data was done using ANOVA and student's *t*-test ($P < 0.05$).
Results: Virological breakthrough (VB) was observed in seven patients (28%) at week 52 of treatment. Histological improvement was observed in 21 CHB patients (84%), despite the emergence of YMDD mutations. Plasma PACAP-38 levels were significantly lower in CHB patients at baseline than in HD (22.62 ± 8.27 pg/mL vs. 65.18 ± 12.61 pg/mL, $P < 0.001$). Significant elevation of plasma peptide levels was observed in CHB patients after the completion of lamivudine treatment period (22.62 ± 8.27 pg/mL vs. 49.60 ± 16.45 pg/mL, $P < 0.001$), even in the subgroup of those who exhibited YMDD variants (21.63 ± 8.57 pg/mL vs. 49.80 ± 15.66 pg/mL, $P = 0.006$).
Conclusion: The elevation of plasma PACAP-38 levels in treated CHB patients, following the lamivudine-induced elimination of viremia, suggests a possible shift to a Th2 immune response, resulting in biochemical and histological remission of liver disease.

P448 Profile of anti-HBcAg response of Th lymphocytes in children with acute hepatitis B

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Objectives: The study aimed at evaluation of the secretory anti-HBcAg response of peripheral blood Th lymphocytes in children with acute hepatitis B.
Methods: The studies included 12 children, aged 10–16 years with acute hepatitis B. T CD4 lymphocytes were isolated from peripheral blood using the biomagnetic technique (Dynal) and rHBcAg stimulated cytokine production (IFN-gamma, IL-4, IL-2, IL-5 and IL-10) estimated by ELISPOT assays (Mabtech; R&D Systems) in 24 h cultures. Studies were performed in the first week of disease and during the recovery.
Results: In the studies, two patterns of Th secretory response were detected. Already in the first week of the disease, the in vitro response to rHBcAg included presence INF-gamma, IL-2, IL-4, IL-5 and IL-10 producing cells, with prevalence of cells producing Il-2 and IFN-gamma. During recovery the number of IL-10 producing cells significantly increased while the numbers of cells producing the remaining cytokines demonstrated a significant decrease.
Conclusion: The presented results point to the Th1-type specific cytokine pattern in the course of acute hepatitis B and the selective prevalence of IL-10 secretion during the recovery.

P449 Subacute liver failure resulting from hepatitis B virus reactivation after chemotherapy for acute myeloid leukemia

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Hepatitis B virus (HBV) reactivation is a well-described complication in cancer patients who receive cytotoxic chemotherapy and may result in fatal liver damage. We report a case of a 45-year-old-man with acute myeloid leukemia (M1) who developed subacute liver failure from reactivation of HBV after chemotherapy. He was an asymptomatic HBV carrier previously. Two-month after chemotherapy, he admitted to the hospital with complain of malaise, anorexia, vomiting, dark urine and icter, and he was hospitalized. On physical examination icter and hepatomegaly was noted. Laboratory findings showed that elevated liver enzyme, very high levels of bilirubine (40 mg/dL), and coagulopathy. HBsAg, anti-HBc and anti-HBe were positive and HBV DNA by PCR was 1.4×105 copy/mL. In addition to supportive therapy, lamuvidine 100 mg/daily was initiated. In 3-month subacute liver failure developed ascites and changes of personality was added to clinical picture despite lamuvidine therapy. This case indicates the importance of HBsAg screening before chemotherapy, and the need to lamuvidine prophylaxis for prevent HBV reactivation in HBsAg positive cases. After reactivation of HBV, unfortunately prognosis may be poor and lamuvidine therapy may be insufficient.

P450 Fatty liver and viral hepatitis

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Objectives: Steatosis is a common histological finding in liver biopsy, associated with some metabolic disorders, but it can be also found in other diseases. The aim of our study was to identify fatty liver in all available liver biopsies and to find associations with viral hepatitis (HBV, HCV).
Methods: We searched liver biopsy database of our Department from 1980 to 2001 (No 3352) for fatty liver (>5% steatosis), and then searched for viral hepatitis infection. We estimated degree of steatosis (5–33% mild, 33–66% moderate and >66% severe) and fat deposition (macrovesicular/microvesicular/mixed). Diagnosis, clinical data, treatment, control liver biopsies and Cole index were recorded and analyzed.
Results: Among 103 pts with fatty liver we identified 36 children (22 boys, 14 girls, aged 1.1–16.1, mean 7.49 years) with viral hepatitis. Mild steatosis was found in 24, moderate in five and severe in seven cases. Histology revealed macrovesicular steatosis in 17 cases and microvesicular in seven (mixed in 12). Seven patients were obese (Cole index > 120%). Two cases of Wilson's disease and single cases of autoimmune hepatitis and operated craniopharyngioma were found. HBV (No 24) was the most common diagnosis. Seventeen patients were found to have HCV infection. Coinfection HBV/HVC was observed in four cases. AlAt activity was increased in 28 pts. Only 7 of 32 examined children had abnormal liver sonograms suggestive of liver steatosis. Both in group of HBV (No 16, 66%) and HCV (No 11, 64.7%) infection the most common was mild steatosis. Six children with HBV had control liver biopsy after finishing interferon therapy. All of them had HbeAg seroconversion and had no steatosis in control biopsy.
Conclusions:
1. Our data indicate that HCV as well as HBV infection may be associated with liver steatosis.
2. Effective treatment of HBV infection can result with disappearing steatosis.
3. There is a limited application of liver ultra sonogram to detect steatosis.

P451 Prevalence of precore mutants in anti-HBe positive chronic HBV carriers in Babol, Iran 1999–2002

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Objectives: Hepatitis B virus precore mutants are associated with highly productive infection in Anti-HBe+ chronic HBV carriers. These chronic HBV carriers are predisposed for developing of active hepatitis. The aim of this study was determination of the prevalence of precore mutants in chronic anti-HBe+ HBV carriers in Babol, Iran.
Methods: This prospective study, was conducted on patients with HbsAg+, anti-HBe+ chronic HBV carriers, from April 1998 to September 2002. In all cases, HBV DNA was assayed by PCR. Data were analyzed by SPSS. Prevalence of precore mutants in males and females was compared by chi-square test.
Results: Of 257 cases of anti-Hbe+ chronic HBV carriers (mean age ± SD, 32.3 ± 11.4 years) HBV DNA was positive in 222 (86.4%) cases. HBV DNA was positive in 136 (87.2%) of 156 males and 86 (85.1%) of 101 females cases ($P = 0.71$).
Conclusions: These results show the high prevalence of HBV precore mutants in Iran.

P452 Histological activity index and its relation to alanine aminotransferase level in precore mutants chronic hepatitis B

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Objectives: Elevated level of alanine aminotransferase (ALT) in chronic HBV carriers is associated with liver damage. The purpose of this study was to assess the relation between elevated levels of ALT with liver injuries.
Methods: Liver biopsy samples from 74 cases of anti-HBe+, HBV DNA+ individuals with ALT more than 40 IU were studied from September 1999 to

September 2002. Histological activity Index (HAI) score more than three with relation to ALT levels both more and less than 1.5 and 2 times of the upper limit of normal (ULN), were compared with χ^2 and fisher's exact tests separately.
Results: A total of 74 cases (63 males and 11 females) with mean age, 31.3 ± 12 years were studied. HAI < 3, between 3 and 8 and more than 8 were seen in 21.7, 63.6 and 14.7%, respectively. Nineteen cases with ALT levels less than 1.5 and 55 cases with ALT levels more than 1.5 times of the ULN, HAI more than 3 was seen in 78.9 and 78.2%, respectively ($P = 0.6$). In 35 cases with ALT levels more than two times of the ULN, HAI more than three was seen in 74%, but in 49 subjects with ALT levels less than two times of the ULN, HAI was seen in 65.3% ($P = 0.29$)
Conclusions: Histological evaluation of liver is suggested for all patients with precore mutants chronic HBV infection with ALT levels more than 40 IU.

P453 Comparative analysis of free and combined fibronectin contents in acute and chronic hepatitis B

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Objectives: The aim of the research was carrying out of comparative analysis of free and combined with circulated immune complexes (CIC) fibronectin (FN) contents in acute and chronic hepatitis B.
Methods: Fifty-seven patients with acute hepatitis B (group 1), 32 patients with chronic hepatitis B (group 2) and 30 healthy persons were investigated. The parameters were determined in plasma at the first day of stay in the hospital and in 1 month. Contents of free and combined FN were determined using ELISA, FN degradation products – using Western blot with the rabbit antibodies to FN, level of CIC – using precipitation with 3% PEG 6000.
Results: In acute hepatitis B, the average contents of free FN was higher than in control group and chronic patients ($t = 6.93$; $P < 0001$; $t = 8.63$; $P < 0001$). Lowering of free FN contents was observed in dynamic ($t = 5.23$; $P < 0001$). The level of CIC at the first investigation in both groups exceeded such in a control group ($t = 6.14$; $P < 0001$; $t = 5.11$; $P < 0005$). In acute hepatitis B the lowering of a CIC level ($t = 3.92$, $P < 0.01$) was observed. It was determined that concentration of combined FN was higher in acute hepatitis patients than in control group and chronic hepatitis ($U = 83.2$; $P < 0.05$; $U = 68.3$; $P < 0.05$). We detected products of a combined FN degradation in the majority of the chronic hepatitis B patients, in group 1 – less than for half of patients and in control group only in 1/6.
Conclusion:
1. In patients with acute hepatitis B the contents of free and combined FN increase in the beginning of disease and decrease in the dynamics simultaneously with a level of CIC;
2. In patients with chronic hepatitis reduced contents of free and combined FN and degradation of combined FN are observed.

		Group 1, $n = 57$		Group 2, $n = 32$	
Parameters	Control group, $n = 30$	1 day	In 1 month	1 day	In 1 month
Free FN (mg/mL)	240.7 ± 36.2	421.1 ± 92.8	284.6 ± 52.2	193.8 ± 48.2	186.4 ± 37.4
CIC (mg/mL)	1.52 ± 0.29	2.31 ± 0.36	1.76 ± 0.34	2.19 ± 0.42	2.22 ± 0.31
Combined FN (mg/mL)	0.25 ± 0.09	0.38 ± 0.1	0.29 ± 0.14	0.19 ± 0.09	0.21 ± 0.09
FN degradation products	5/30	22/57	12/57	24/32	26/32

P454 Acute hepatitis due to brucellosis

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Objectives: Brucellosis is an acute or chronic infectious disease involving several organs. Liver function tests are usually only slightly elevated. Acute hepatitis due to brucellosis is quite rare. We report two cases of acute hepatitis due to *Brucella* infection.
Case 1: A 37-year-old woman, a clinical microbiology laboratory worker, presented with fever, headache, arthralgia on knee, anorexia, and nausea. Temperature was 37.5°C, pulse 80/min. Physical examination was normal. WBC 4000/mm^3 with 50% lymphocytes, 40% neutrophils, 10% monocytes, CRP 10.5 mg/L ($N < 5$ mg/L), total bilirubin 0.61 mg/dL, ALT 552 IU/mL, AST 473 IU/mL, alkaline phosphatase 352 IU/mL. Prothrombin time was normal. Anti-HBs was (+); anti-HCV and anti-HAV IgM were (–). Agglutination tests (Rose Bengal and Wright; 1/1280) were (+). *Brucella* sp. was isolated from blood cultures on the fifth day. Doxycycline and rifampicin were given for 6 weeks. On the day 4, the fever subsided, and on day 15, liver enzymes returned to normal values.
Case 2: A 20-year-old man was admitted with polyartralgia, difficulty in walking and morning stiffness. He had been first treated as ankylozing spondylitis with corticosteroids and salazopryin. He had responded to the treatment clinically, the drugs had been tapered and discontinued within 3 months. Then fever and diarrhea developed. Three days later, he had been initiated ciprofloxacin but not responded. On admission, temperature was 39°C, pulse 80/min. Physical examination was normal. WBC 6000/mm^3 with 50% lymphocytes, 40% neutrophils, 10% monocytes, CRP 10.5 mg/L, total bilirubin 0.61 mg/dL, direct bilirubin 0.17 mg/dL, ALT 392 IU/mL, AST 328 IU/mL, ALP 232 IU/mL. HBsAg, anti-HBcIgM, anti-HCV and anti-HAV IgM were (–). Agglutination tests (Rose Bengal and Wright; 1/1280) were (+). *Brucella* sp. was isolated from blood cultures on the fifth day. Doxycycline and rifampicin were given for 6 weeks. The treatment was interrupted due to drug eruption and diarrhea. After 10 days, rifampisin and streptomycin were started. Fever subsided within 1 week, liver enzymes returned to normal within 3 weeks.
Conclusion: Brucellosis may be presented with elevated liver enzymes and should be considered in the differential diagnosis of acute hepatitis in the countries where brucellosis is endemic.

P455 Hepatitis A virus infection in adults

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Introduction: Hepatitis A viral infection (HAVi) has a worldwide distribution and causes clinical disease more frequently in children than adults. The severity of the disease ranges from subclinical infection, classic clinical infection, and very rarely to fulminant hepatitis and death. No transition to chronic active hepatitis is observed, but in more than 15% relapse occurs.
Objectives: The aim of our study is to present our experience with HAVi in adults and to show that the course of this infection can be severe with prolonged clinical course and relapsing forms.
Materials and methods: During a period of 5 years, 325 patients with diagnosis viral hepatitis, were treated in our department. Two hundred five patients were with HAVi (63%). Clinical signs and symptoms of liver disorders, biochemical markers (bilirubin total, ALAT, ASAT, gamma GT, alkal, phosphatase, fibrinogen, CRP, total proteins) had been analyzed in 46 patients aged 35–70 years. All of the patients presented a serious and long course of the disease with high levels of bilirubinemia (110–458 mmol/L, and serum increased transaminases activity (ALAT: 10.804–38.098 nKat/L; ASAT: 5.986–29.831 nKat/L). Signs of cholestasis were present in 19 patients, acute cholecystitis and choleangitis had been present in 5 patients. Fulminant form with dead developed in one 23-year-old student. Our patients were treated from 32 to 45 days in our department. Normalizing of the biochemical changes was in 70%. One-month later abnormal ALAT and ASAT were registered in nine patients. Relapse was in two patients with alcohol abuse.
Conclusion: HAVi is a great and significant problem in our region. The acute HAVi in adults is severe and with risk of complications, prolonged clinical course and relapses.

P456 Sero-epidemiological characteristics of hepatitis A in the region of Bitola, Macedonia

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Objectives: Infection with Hepatitis A virus (HAV), remains a significant health problem worldwide. The aim of our study is to present the seroepidemiological characteristics of HAV in our region for the period of 3 years (1999–2002).
Material and methods: We have analyzed 398 sera, which were obtained from 211 men and 187 women. They were tested for anti-HAV IgG and anti-HAV IgM antibodies by ELISA (Abbot-USA). Children under 2 years of age and blood-transfusion recipients were excluded from the study. According to the place of living, 152 persons were living in our town, and the other 246 persons were from the surrounding villages.
Results: The persons examined were assigned to two age groups (A) 167 children 2–15 years old, and (B) 231 adults 16–70 years old. In group A 68 (41%) persons were IgG positive and among them 21 (12.5%) were seropo-

sitive to IgM antibodies also. In group B 176 (76%) subjects were anti-HAV IgG positive and only 15 (6.5%) developed acute hepatitis and were IgM positive.
Conclusions: The high percentage (59%) of children negative for anti-HAV IgG antibodies coupled with the fact that most of the adults (76%) had developed protective immunity against HAV suggests that our area has shifted from high endemic to intermediate-low endemic status for hepatitis A infection. The great number of susceptible children is a potential risk for the development of a serious prolonged icteric infection during the adulthood. In this context, immunization against HAV might be considered to be performed during the first years of life.

P457 Duration of seropositivity of IgM anti-HAV after acute viral hepatitis A infection

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Viral hepatitis A infection is still a global public health care problem specially in some certain parts of the world. Like many other countries, this disease, which is caused by the hepatitis A virus (HAV), is endemic in some regions of Turkey with poor hygienic conditions. The exact diagnosis of acute hepatitis A can be made easily by the finding of IgM anti-HAV, which is necessary for the diagnosis of acute hepatitis A, in the serum of the patients. It can be detectable serologically in serum by the time of onset of disease in a patient with either clinical symptoms or biochemical evidence of the acute hepatitis. In this prospective study, we have planned to investigate the duration of IgM anti-HAV seropositivity in patients with acute hepatitis A. The study was performed at SSK Izmir Teaching Hospital, which is a social security hospital with 650 beds, between November 1995 and May 2001. A total number of 68 patients were enrolled in the study who applied to the Department of Infectious Diseases and Clinical Microbiology, presenting with typical clinical symptoms and laboratory findings and whom IgM anti-HAV were detected for the purpose of exact diagnosis of acute hepatitis A. These patients were then asked to apply for regular controls and serum measurements of IgM anti-HAV were performed for every 3 months. All of the serologic testiness for IgM anti-HAV were performed by EIA. The results obtained in the end of the study were as follows; IgM anti-HAV had disappeared in the sera of four (5.9%) patients after 3 months, in six (8.8%) patients in the second quarter, 38 (55.9%) in the third quarter, 13 (19.1%) in the fourth quarter and two (2.9%) in the fifth quarter. The duration of the seropositivity in the remainder five patients were 21 months in two (2.9%) patients, 33 months (1.4%), 36 months (1.4%) and 46 months (1.4%) in one patient, respectively. Finally, we can conclude that in the sera of the majority of patients (70.6%) with acute hepatitis A, the IgM anti-HAV titters were found to be below detectable levels by EIA tectonics after 9 months, while the same duration can be as long as 46 months after the onset of the infection.

P458 Survey of protection from hepatitis A in the last guide school students in Yazd

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Objective: Hepatitis A, a viral disease, is spread by the fecal-oral route. Anti-hepatitis A IgM is detectable in serum 45–60 days after the onset of symptoms. Titers of anti-HAV from IgG class rise with convalescence and the antibody usually persists for many years. Recovery from infection is associated with lifelong immunity.
Methods: In this cross-sectional study. Anti-HAV from IgG class against HAV was examined among 226 students include 113 males and 113 females in Yazd guide schools by ELISA method. Variables such as sex, age, parent's job, parent's education, and economic status also were purposed.
Results: Mean age of individuals was 14.17 years.18 (8 males and 10 females) out of total 226 had not IgG against the virus, which is considered as nonimmune. There was no significant correlation between sex, parent's job and parent's education and immunity to HAV.
Conclusion: Hepatitis A infection usually occurs during early life years in Yazd and administration of normal serum immunoglobuline for preventing HAV infection in adults older than 14-year-old is not recommended.

P459 The study of prevalence of hepatitis B and C markers in patients with hepatocellular carcinoma

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Objectives: The aim of this study was to evaluate the prevalence of chronic HBV, HCV infections in Iranian patients who were known cases of H.C.C.
Methods: During the years 1999–2002, patients who were known cases of H.C.C, serologic tests of viral markers (HBV, HBV DNA, HCV, HDV) were examined and the results were recorded.
Results: Twenty-three patients (16 males and 7 females) with a mean age of 50 and a range of 40–60 were included in this study. Twenty-one (91%) were chronic carriers of HBV, amongst whom 12 (62%) patients were HBV DNA positive, 2 (9%) had no chronic infections of HBV, HCV. Three (13%) had positive markers of HBV, HCV, but in none of them was only HCV marker present. Twelve (52%) of HBV patients had super infection with HDV.
Conclusion: Chronic HBV infection was the most common risk factor of H.C.C. This result is expectable because in our country the prevalence of chronic HBV is high.

P460 Association between seropositivity of antibodies against *Helicobacter pylori* and hepatitis A virus in Iran: evidence regarding shared risk factors of transmission

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Objective: There is a hypothesis that infections of *Helicobacter pylori* (*H. pylori*) and hepatitis A virus (HAV) have a strong association, suggesting similar modes of transmission. The aim of this study was to know whether the hypothesis was true in Iran and to determine the prevalence of coincidence of both infections in the same individual.
Methods: Subjects were 372 healthy individuals (age range of 3–81 years) who underwent health check program in Sari, Iran. Blood samples and questionnaire data were collected. Serum sample from each subject was analyzed for *H. pylori* IgG antibody by enzyme linked immunosorbent assay and for HAV IgG antibody by microparticle enzyme immunoassay. The association between seropositivity of anti-HP and anti-HAV antibodies was examined for the statistical significance by χ^2 and Fisher exact tests. Multiple logistic regression analysis was used to derive multivariate adjusted Odds ratio (ORs) and it's 95% Confidence Interval (CI).
Results: Thirty-one subjects (8.3%) below the age of 20 were seropositive for *H. pylori* and 47 (12.6%) were seropositive for HAV. In adults, 232 (62.3%) were seropositive for *H. pylori* and 274 (73.6%) were seropositive for HAV. Two hundreds and 87 subjects (77.15%) were seropositive for both *H. pylori* and HAV infections in the same time ($P = 0.0001$, 95% CI = 1.24–2.96,OR = 2.12). The prevalence of coincidence of both infections was 7.2% among children, 59.6% among adults, 19.3% in male and 41.4% in female. Forty four (11.8%) subjects were seronegative for both *H. pylori* and HAV ($P < 0.0001$). One hundred and eight (29%) subjects were seronegative for *H. pylori* only and 52 (13.9%) were seronegative for HAV only. Anti-HAV seroprevalence was (16.6%) among anti-*H. pylori* seronegative and (1.3%) among anti-*H. pylori* seropositive individuals. The association between anti-HAV and -*H. pylori* seropositivity were statistically significant after adjustment for age and sex through multiple logistic regression analysis ($P = 0.003$, OR = 2.12, 95% CI = 1.24–2.96). There was a significant relation between coincidence of both infections and age ($P = 0.002$, OR = 6.9, 95% CI = 4.07–11.85). Length of schooling and occupation as surrogate for socio-economic status ($P = 0.0001$, OR = 1.15, 95% CI = 0.61–2.16).
Conclusion: There was a strong association between seropositivity of *H. pylori* and HAV antibodies in Iran which reflects a relation between routes of transmission for HAV and *H. pylori*.

Mechanisms of antimicrobials

P461 Stable changes in antibiotic sensitivity and cell wall structure associated with a transient cell wall deficient phenotype in *Staphylococcus aureus*

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Objectives: Cell wall deficient bacteria (CWDB) have been associated with a number of disease processes such as burn site infections and sarcoidosis, but little is known about the triggers that cause the loss of cell wall, in vivo, or the consequences of cell wall deficiency on bacterial metabolism. Our objectives were to characterize the changes in antibiotic sensitivity and cell wall structure that occur when *Staphylococcus aureus* cells undergo a transient cell wall deficient phenotype.
Methods: We induced cell wall deficient variants of *S. aureus*, on media with high osmotic potential, in the presence of sublethal levels of penicillin. Antibiotic sensitivity profiles were determined under BSAC guidelines for disc diffusion tests; enzyme sensitivity and mass spectrometric methods were used to analyze cell wall structure.
Results: CWDB were resistant to beta-lactams, stained Gram-negative, had lost the typical staphylococcal cell arrangement and had an indistinct margin. When cells were allowed to recover their wall by passage in the absence of penicillin, they displayed stable resistance to beta-lactam antibiotics and altered sensitivity to several other classes of antibiotic. In this revertant state, they also displayed decreased sensitivity to lysis by lysostaphin and an altered signature on intact cell matrix assisted laser desorption/ionization time of flight mass spectrometry.
Conclusions: *S. aureus* cells which have been induced to transiently lose their cell wall, in the presence of penicillin, acquire stable resistance to beta-lactams and have altered sensitivity to several other classes of antibiotics. We hypothesize that the change in antibiotic sensitivity profile is due in part to altered structure of the regenerated bacterial cell wall.

P462 Penicillin binding protein profiles of *Aeromonas veronii* bv. *sobria*

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Objectives: Members of the genus *Aeromonas* are emerging pathogens that pose a threat especially to immunocompromised patients and patients with severe diseases. To be able to map out effective therapeutic strategies it is important to understand the mechanism of action of the available antibiotics and also to understand the mechanisms of resistance towards these substances. Beta-lactam antibiotics have been shown to bind to and inhibit penicillin binding proteins (PBPs) causing a disruption of the cell wall metabolism and eventually cell lysis. Until now nothing is known about the PBPs of *Aeromonas veronii*. We identified the PBPs in membrane preparations of *A. veronii* bv. *sobria* by using biotinylated ampicillin, in order to create a basis for further studies of the cell wall metabolism which might also be linked to beta-lactamase induction.
Methods: Biotinylated ampicillin was used to specifically mark the PBPs in membrane preparations of *A. veronii*. After separation by SDS–PAGE the proteins were transferred onto a pvdf membrane by Western blotting. Unspecific binding sites were blocked with 5% milk powder. The membrane was flooded with a streptavidin-peroxidase-conjugate which binds specifically to biotinylated ampicillin and which was detected by ECL reagents on an X-ray film.
Results: Using biotinylated ampicillin at a concentration of 40 μg/mL nine bands were detected with molecular masses between 20 and 100 kDa. A sample containing no biotinylated ampicillin was used as a negative control. There was one band in this sample which must be considered to bind nonspecifically to streptavidin.
Conclusions: Eight PBPs with molecular masses between 20 and 100 kDa were identified in membrane preparations of *A. veronii* by using biotinylated ampicillin. This finding will be the starting point for further studies of the role of PBPs in cell wall metabolism of *A. veronii* using deletion mutants that miss one of the eight PBPs.

P463 Inoculum effects with cephalosporins in *Plesiomonas shigelloides* are due to formation of enormous filaments

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Objectives: The species *Plesiomonas shigelloides* is mainly associated with gastrointestinal diseases. However, more severe complications as septicaemia and meningitis in patients with preexisting health problems have also been described. Appropriate antibiotic therapy is necessary. Therefore, detailed knowledge about resistance mechanisms in this species is needed. *P. shigelloides* has been described as an organism showing pronounced inoculum effects in susceptibility testing for cephalosporins. We investigated if these inoculum effects are due to the production of beta-lactamases.
Methods: Three strains of *P. shigelloides* were used (clinical and environmental isolates). Beta-lactamases were characterized by activity tests, SDS–PAGE and isoelectric focusing. MIC values for 15 cephalosporins were determined by microdilution methodology using inocula of 1×10^5 CFU/mL and 1×10^6 CFU/mL. The morphology of cells was determined by light microscopy. One *E. coli* and one *P. stuartii* strain were used as comparative strains for microscopy after cephalosporin treatment. For one *P. shigelloides* isolate kill kinetics with 0125 mg/mL cefpodoxime were determined using batch cultures with inocula of 1×10^5 CFU/mL and 1×10^6 CFU/mL.
Results: Two *P. shigelloides* strains were shown to be beta-lactamase positive. They harboured a constitutively expressed noninducible enzyme with a molecular weight of 29 kDa and pI values of 4.9 and 5.3. The cephalosporin susceptibility of the *P. shigelloides* strains including the lactamase-negative isolate showed a dependency on the inoculum size. The MIC for most cephalosporins increased 64-fold or more with the higher inoculum. Examination of cells from cavities of the MIC microtitre plates with inocula of 1×10^6 CFU/mL revealed a very strong filamentation for most cephalosporins. The length of the filaments ranged from 100 μm up to 2 mm. Far shorter filaments were seen for *E. coli* and *P. stuartii*. The kill kinetic with cefpodoxime for *P. shigelloides* revealed similar kill curves for both inocula with an elimination of viable cells after 8–10 h.
Conclusions: The inoculum effect seen with cephalosporins is not due to the possession of a beta-lactamase but can be attributed to extensive filamentation of the single cells. This should be considered when interpreting the MIC data. Even if cephalosporins kill the cells during infection there might be a deleterious effect due to increased LPS production caused by excessive filamentation.

P464 A quantitative assessment of dose-dependent beta-lactam induced filament-formation in Gram-negative bacteria

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Objectives: Beta-lactam-antibiotics block Penicillin-binding-proteins (PBPs), located in the outer membrane of Gram-negative bacteria. Previously, we showed that binding to PBP-1 leads to a quick bacterial lysis, and release of small amounts of endotoxin (ET). Binding to PBP-3 causes formation of long strands of bacterial material (filaments). After filament lysis, large amounts of ET are released, and this might cause an excessive cytokine-response with a further deterioration in clinical course. Beta-lactams have specific binding affinities for the different types of PBP, often in a dose-dependent manner. For ceftazidime and cefotaxim, PBP-3 binding occurs in low dosages, PBP-1 binding in high dosages. Aim of our study was to assess the concentrations at which these changes in binding-affinity occur, using several Gram-negative strains.
Methods: Three bacterial strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 en *K. pneumoniae* ATCC 43816) were tested with ceftazidime and cefotaxim at 9 dose-levels (0, 0.1, 1, 5, 10, 20, 30, 40 and 50 mg/L). Overnight cultured strains were incubated during 4 h. For each specimen, Gram-stains and bacterial counts were performed.
Results: The dose-level at which affinity changed from PBP-3 to PBP-1, differed per combination. For ceftazidime, concentrations exceeding 30 mg/L prevented filament formation in all strains tested. Using cefotaxim, these levels had to be higher than five and 10 mg/L for *E. coli* and *K. pneumoniae*,

respectively. However, for pseudomonas, concentration-levels above 40 mg/L were required. These minimum concentrations were not related to the MICs for the respective bacteria and antibiotics. In pseudomonas-cultures, filament-formation did not occur at sub-MIC dose-levels, in contrast to *E. coli* and *K. pneumoniae* cultures.
Conclusions: The changes in PBP-binding pattern are specific for each combination of antibiotic and bacterial strain, and are independent of MICs for these combinations. Given the possible negative effects of filament formation, dose levels should be kept at relatively high levels during the first hours of treatment, particularly when using beta-lactams with a dose-dependent affinity for different PBPs.

P465 Induction of fibronectin-binding proteins by subinhibitory levels of ciprofloxacin in quinolone-resistant *Staphylococcus aureus* occurs independently from the sigma B transcription factor activity

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Objectives: We previously reported up-regulation of fibronectin-binding proteins (FnBP) and promotion of fibronectin-mediated adhesion by sub-MICs of ciprofloxacin (CFX) in quinolone-resistant (QR) topoisomerase IV-gyrase double mutants of *S. aureus* NCTC strain 8325. This study evaluates the potential influence of the transcription factor Sigma B (SigB) in the CFX-triggered up-regulation of FnBP, using mutants of QR strain RA1 expressing different levels of SigB activity.
Methods: The rsbU 8325-derived strain RA1 exhibits low levels of SigB activity. Strain TE1 is a transposon-inactivated *sigB* knockout mutant of RA1. Strain TE2 is a rsbU + *cis* complemented derivative of RA1 whose SigB activity is restored. Surface display of FnBP was evaluated by bacterial adhesion and flow cytometry. Steady-state levels of fnbA and fnbB mRNAs were assayed by quantitative RT-PCR (TaqMan) and correlated with mRNA levels of sigB and SigB-specific target gene *asp23*. CFX-promoted responses were tested at 4 mg/L (1/8 MIC for each strain) of CFX.
Results: Following growth in CFX-free medium, the different SA strains showed a positive correlation between their FnBP surface display and their SigB activity. Compared to the *sigB* mutant TE1, fibronectin-mediated adhesion of rsbU and rsbU ± restored strains RA1 and TE2 increased by 50 and 100%, respectively. Steady-state sigB mRNA by the rsbU ± restored strain TE2 was 5–6-fold higher than that of its rsbU parent RA1. Steady-state mRNA of the SigB-dependent target gene *asp23* was seven-fold higher in the rsbU ± restored strain TE2 than in the rsbU parent RA1, and 100-fold higher than in the *sigB* mutant TE1. In contrast, after growth in the presence of CFX, adhesion of strains TE1, RA1, and TE2, increased by 83, 60, and 60%, respectively, compared to CFX-free medium. CFX-exposed strains TE1, RA1, and TE2 showed 2- and 3-fold increases in fnbA and fnbB steady-state mRNA levels, respectively, regardless of their different SigB activities.
Conclusions: SigB can up-regulate FnBP surface display and bacterial adhesion in QR strains but is not required for the CFX-promoted response that likely involves another independent stress response pathway.

P466 Global distribution of serotypes in *Streptococcus pneumoniae* isolated from pediatric patients in the PROTEKT 1999–2000 surveillance study

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Background: The 7-valent pneumococcal conjugate (7-V) vaccine contains polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. Cross-coverage is thought to extend to other serotypes (ST) within the same serogroup (SG) although this may differ from one SG to the other. The aim of this study was to determine the global distribution of *S. pneumoniae* STs in a large population of phenotypically and genotypically defined pediatric isolates.
Methods: *S. pneumoniae* isolates were collected worldwide from children aged ≤14 years with community-acquired RTIs. MICs were determined at a central laboratory using the NCCLS broth microdilution method and interpreted using NCCLS breakpoints. Macrolide resistance (MacR) mechanisms were determined by PCR in erythromycin-resistant isolates (MIC ≥ 1 mg/L). Serotyping was performed using antisera obtained from the Statens Serum Institute Reference Laboratory (Copenhagen, Denmark).
Results: A total of 775 isolates of *S. pneumoniae* were typed. Overall, 59.7% of STs and 74.8% of SGs were covered by 7-V; however, there was considerable variation between various subpopulations (location, age, site of infection). MacR, and penicillin resistance (PenR) were high in the vaccine STs. MacR mechanisms differed in prevalence between the various STs. The SGs of most of the isolates outside of the coverage of the vaccine belonged to either SG 3, 11 or 15. Thirteen out of 24 SG 15 and 2/24 SG 11 isolates were multiresistant (MacR, PenR, tetracycline-resistant). SG 3 is considered one of the more virulent SGs and ranked as the fifth most prevalent SG in the study. Six of the 41 SG 3 isolates were MacR (erm(B)-mediated) and TetR (but PenS). Telithromycin was highly active against all serotypes (MIC50 0.015 mg/L, MIC90 0.25 mg/L and range 0.002–1 mg/L).
Conclusions: Globally, three SGs of *S. pneumoniae* were found to represent the major proportion of isolates outside of the 7-V vaccine coverage. As with STs covered by the vaccine, MacR and PenR in these isolates were high. MacR and TetR in the virulent SG3 strains is unexpected and of concern. Telithromycin demonstrated excellent activity against all STs in this pediatric population. These data suggest that the use of telithromycin in pediatric populations vaccinated with 7-V may be more effective than macrolides or penicillins in limiting serotype replacement.

P467 The fungicidal activity of eugenol on *Candida* spp. results from a primary lesion of the cell membrane

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Objectives: Eugenol is the major constituent (85.3%) of the essential oil of *Syzygium aromaticum* (*Eugenia caryophylla*). The oil has high antimicrobial activity, similar to eugenol, although its mechanisms of activity remain yet unclear.
Material and methods: The minimal inhibitory concentration (MIC) and minimal fungicidal concentrations (MFC) to essential oils and eugenol were determined for 10 *Candida* strains (three *C. albicans*, two *C. tropicalis*, two *C. glabrata*, one *C. krusei*, one *C. guillermondii*, one *C. parapsilosis*). Germ tube formation in presence of subinhibitory concentrations was also assessed. Additionally, following a short incubation of the yeast cells with eugenol, flow cytometry analysis was performed after staining with propidium iodide (PI, a fluorescent probe that selectively stains cells with severe membrane damage). Studies of interactions between eugenol and fluconazole using the Checkerboard method (1) were also performed.
Results: MIC and MFC were similar, ranging between 0.31 and 1.25 µL/mL. Germ tube formation was inhibited at concentrations lower than those which inhibited growth. Following a short incubation period PI penetrated most of the yeast cells, meaning that the cell membrane was disintegrated by the drug. No synergistic or antagonistic effects were found between eugenol and fluconazole.
Conclusions: Eugenol shows a fungicidal activity on *Candida* spp. by lesion of the cell membrane. Our data supports the use of *Syzygium aromaticum* (*Eugenia caryophylla*) for topical use in mucocutaneal Candidosis, as an isolated agent or in association with fluconazole, in case of being devoid of side-effects. One-Scott EM, Tariq VN, McCrory RM. Demonstration of synergy with fluconazole and either ibuprofen, sodium salicylate, or propylparaben against *Candida albicans* in vitro. Antimicrob Agents Chemother 39 : 2610–2614, 1995.

β-lactams, penicillins and cephalosporins

P468 In vitro activity of ertapenem against anaerobes isolated from the respiratory tract

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Objectives: To test the activity of ertapenem and other antibiotics against anaerobes isolated prospectively from the lower and upper respiratory tracts, and to compare their susceptibility with that of anaerobic isolates from other body sites.
Methods: Fifty-three anaerobic isolates from the lower (21) and upper (32) respiratory tracts (RT), as well as 49 isolates from various other body sites (N-RT) were tested with *E*-tests against penicillin (PEN), clindamycin (CLI), amoxicillin/clavulanic acid (AUG), moxifloxacin (MOX), ceftriaxone (CTR) or cefoxitin (CFX) and ertapenem (ERT).
Results: MIC 50 and MIC 90 values (mg/L) are presented in the table below.

		PEN	CLI	AUG	MOX	CTR	CFX	ERT
RT	MIC 50	0.064	0.023	0.047	0.094	0.19	NA	0.023
	MIC 90	>32	12	0.38	2	>32	NA	0.125
N-RT	MIC 50	4	1	0.25	0.5	NA	4	0.19
	MIC 90	>32	>256	1	16	NA	24	1

The higher susceptibility of respiratory tract isolates is mainly due to the different distribution of isolated species: only three isolates belonged to the *Bacteroides fragilis* group (BAF), while the others were 26 other Gram-negative bacilli (OGNB), 17 cocci (COC) and seven nonsporeforming Gram-positive bacilli (NSFGPB). The NRT isolates were 22 BAF, 5 OGNB, 7 *Clostridium* spp., 9 NSFGPB and 6 COC.
Conclusions: This study confirms the excellent antianaerobic activity of ertapenem against anaerobic isolates from the respiratory tract: the MIC 90 values were lower than that of any antibiotic commonly used in respiratory tract infections. All isolates were susceptible, as it was also the case for AUG, while, respectively, 22.6% were resistant to PEN, 18.9% to CTR, 11.3% to CLI and 1.9% to MOX.

P469 Single i.v. doses of ertapenem are not associated with clinically significant QTc prolongation

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Objectives: Ertapenem is a parenteral once daily beta-lactam antibiotic. QTc prolongation has been associated with an increased incidence of a potentially fatal arrhythmia, Torsades de Pointes. While studies in cats with high doses of ertapenem did not reveal any significant QTc prolongation, the purpose of this study was to evaluate the effects of peak drug concentrations on QTc interval in healthy subjects. In order to provide a safety margin, instead of a 1 g dose, the dose approved for clinical use by worldwide regulatory agencies, a 2 g i.v. dose was administered in this study.
Methods: Twenty-four subjects, 10 men and 14 women, received a single 2 g i.v. dose of ertapenem ($N=20$) or placebo ($N=4$), infused over 30 min, in a randomized, double-blind, parallel-panel study. ECG measurements and assay of plasma concentrations of ertapenem were obtained at predose, end of infusion, and 1.5 h from the start of infusion. QTc intervals were machine- and manually calculated using Bazett's equation. QTc prolongation was assessed based on regulatory guidelines where a QTc interval change of >60 ms over baseline or an absolute value >450 ms in men or >470 ms in women are clinically significant.
Results: No subject met the criteria for clinically significant QTc interval prolongation. No postdose QTc intervals were >30 ms over baseline for machine-calculated values. For manually determined QTc intervals, two subjects out of 20 who received ertapenem and one out of four who received placebo had increases in QTc interval of borderline significance (>30 ms but <60 ms). The mean concentration of ertapenem was 282.9 μg/mL at 0.5 h and 166.9 μg/mL at 1.5 h postdose, consistent with previous studies.
Conclusion: Single 2 g i.v. doses of ertapenem are not associated with clinically significant prolongation of the QTc interval in healthy subjects.

P470 Comparative activity of ertapenem and other antibiotics against recent Belgian isolates of *Enterobacter aerogenes*

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Objectives: Ertapenem (ERTA) is a new once-a-day parenteral carbapenem with enhanced activity against Enterobacteriaceae, gram-positive and anaerobes. This study aimed to compare the activity of ERTA with that of imipenem (IMI), meropenem (MERO) and other comparators against 285 *E. aerogenes* isolates collected in 61 Belgian hospitals during the period 2000–2001 as part of a national survey.
Methods: MICs were performed by *E*-test and by agar dilution following NCCLS guidelines (2001).
Results: The activity of ertapenem and other comparators (MIC50 and MIC90 in μg/mL, percentage strains not susceptible at NCCLS susceptibility breakpoints) is shown in the table.

Antibiotics	Range	MIC_{50}	MIC_{90}	% not susceptible (NCCLS breakpoint)
Ertapenem	≤0.03–32	0.25	0.5	2.5
Imipenem	≤0.03–32	0.25	1	2.5
Meropenem	≤0.03–32	0.06	0.12	0.3
Cefepime	0.03–64	1	8	6.7
Temocillin	0.12–128	16	32	16.3
Ceftazidime	0.12–>256	>256	>256	86.2
Ciprofloxacine	0.03–128	32	64	86.6

The carbapenems appeared as the three most active compounds against *E. aerogenes* isolates with MERO > ERTA = IMI in terms of intrinsic activity based on the MIC50 value. No difference in activity was noted for ERTA nor for the other carbapenems against ESBL-positive vs. ESBL-negative *E. aerogenes* strains. On the other hand the activity of most other antibiotics was markedly reduced against ESBL-positive isolates.
Conclusions: ERTA is a new parenteral beta-lactam antibiotic demonstrating high potency against multiresistant *E. aerogenes* isolates irrespective of ESBL production. Owing to its interesting pharmacokinetic properties, ERTA may prove as an interesting adjunct to the limited number of agents currently available for the treatment of infection caused by multiresistant *E. aerogenes*.

P471 Activity of ertapenem against *S. pneumoniae* with reduced penicillin susceptibility

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Objectives: To compare the susceptibility of *S. pneumoniae* with reduced penicillin-susceptibility to commonly used respiratory *β*-lactam antibiotics and to ertapenem
Methods: 200 *S. pneumoniae* isolates were randomly selected from the Belgian national reference collection. All isolates were from invasive infections and isolated in 2001 and 2000. Seventy-five isolates had a Pen MIC > 1 (peni-R), 100 had a Pen MIC > 0.125 and <1 (peni-I) and 25 strains had a Pen MIC < 0.125 (peni-S). All strains were tested against amoxicillin, ertapenem, cefotaxime, cefuroxime and imipenem. The method used was *E*-test on Mueller-Hinton agar supplemented with blood. *S. pneumoniae* ATCC 46916 was used as control strain.
Results: see Table 1.

Table 1

	Peni-S		Peni-I		Peni-R	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Imi	0.006	0.012	0.094	0.125	0.125	0.5
Amoxy	0.012	0.016	0.38	1	0.75	1.5
Cefur	0.016	0.094	1.5	2	2	3
Ceftria	0.016	0.032	0.25	0.5	0.5	0.5
Erta	0.012	0.023	0.19	0.38	0.25	0.5

Conclusion: Increasing MIC values for penicillin and amoxicillin lead to increasing MIC values for ertapenem, but resistant isolates were not yet found. The MIC ratio's for ertapenem vs. the other antibiotics tested change little with increasing MIC-values. On the basis of the MIC distribution curves and MIC50 values, it appears that ertapenem is two-fold more active than ceftriaxone, two to four-fold more active than amoxicilline and two-fold less active than imipenem.

P472 Comparative in vitro activities of panipenem against clinical isolates of aerobic and anaerobic bacteria

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Objective: Panipenem (PAPM) has a broad-spectrum antibacterial activity and used for treatment of blood stream, respiratory and other infections. In this study in vitro activities of PAPM against clinical isolates of aerobic and anaerobic bacteria were compared to those of other drugs.
Methods: Aerobic bacteria were isolated in 2001 and anaerobic bacteria in 2000–2001 from patients at a tertiary-care hospital in Korea. Antimicrobial susceptibility was tested by NCCLS agar dilution methods. For the interpretation of MIC of PAPM, imipenem (IPM) breakpoint was used.
Results: MIC ranges of PAPM were similar to those of IMP and meropenem (MPM) for all of the aerobic and anaerobic bacteria tested. MIC ranges of PAPM were: ≤0.125 mg/L for methicillin-susceptible (MS) *Staphylococcus aureus*, MS coagulase-negative staphylococci, group A and B streptococci, pneumococci, and *M. catarrhalis*; ≤2 mg/L for *H. influenzae*; ≤1 mg/L for *E. coli*, *K. pneumoniae*, *K. oxytoca*, *C. freundii*, *E. aerogenes*, *P. mirabilis* and *P. vulgaris*; ≤2 mg/L for *E. cloacae* and *M. morganii*; 0.12–8 mg/L for *S. marcescens*; 0.03–16 mg/L for *Providencia* spp.; 0.12–128 mg/L for *A. baumannii*; 0.25–128 mg/L for *P. aeruginosa*. MIC ranges of PAPM were = <1 mg/L for *Peptostreptococcus* spp.; ≤0.06 mg/L for *C. perfringens*; ≤8 mg/L for *B. fragilis*; ≤32 mg/L for *B. thetaiotaomicron*. All of the gram-positive cocci and *M. catarrhalis* were interpreted susceptible to PAPM. All of the *H. influenzae* were susceptible to PAPM. All of the Enterobacteriaceae except 8% of *Providencia* spp., and except 27% of *A. baumannii and* 27% of *P. aeruginosa* were susceptible to PAPM. None of *Peptostreptococcus*, *C. perfringens* and *B. fragilis* was resistant to PAPM. Resistance rate of *B. thetaiotaomicron* to PAPM was 7%.
Conclusion: In vitro activity of PAPM was similar to those of IPM and MPM. All of the isolates were susceptible to PAPM except some isolates of *Providencia* spp. and *B. thetaiotaomicron*, and significant proportion of *A. baumannii and P. aeruginosa*, suggesting usefulness of PAPM for the treatment of most infection including mixed ones.

P473 Interpretive breakpoints for susceptibility testing of bacteria against penicillin G/Sulbactam in the disk diffusion test according to the German DIN Standard 58940

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Objectives: For a specific beta-lactam/beta-lactamase inhibitor combination, the German Institute of Standards (DIN) usually defines the same minimal inhibitory concentration (MIC) breakpoints as for the beta-lactam alone. For penicillin G (±sulbactam) the breakpoints are ≤0.125 mg/L (susceptible), 0.25–1 mg/L (intermediate), and ≥2 mg/L (resistant). Sulbactam is added to penicillin G at a fixed concentration of 8 mg/L. Here we present data from a multicenter study in order to determine inhibition zone diameter (IZD) breakpoints for penicillin G/sulbactam.
Methods: In 10 laboratories, MICs and IZDs according to DIN Standard 58940 were determined in parallel for 1324 fresh clinical isolates, mainly of *Staphylococcus aureus* ($n = 352$), streptococci ($n = 90$), *Enterococcus faecalis* ($n = 181$), *Acinetobacter* spp. ($n = 35$), *Escherichia coli* ($n = 305$) and other Enterobacteriaceae ($n = 333$). Test disks were loaded with 10 micrograms each of penicillin G and sulbactam. To determine IZD breakpoints, we first performed a linear regression analysis and subsequently the error rate-bounded method described by Metzler and de Haan (J Infect Dis 1974, 130, 588–594).
Results: The statistical analysis of the MIC and IZD data revealed a correlation coefficient (r) of 0.5597. In cases where r is below 0.85, the DIN requests to apply the error rate-bounded method. Using this method, the following breakpoints were determined for interpreting IZDs: ≤20 mm (resistant), 21–25 mm (intermediate), and ≥26 mm (susceptible). Both the rates of very major and major errors were at an adequate low level of 0.6 and 2.5%, respectively. The rate of minor errors was 21.1%.
Conclusion: Penicillin G/sulbactam disks loaded with 10/10 micrograms are suitable for routine susceptibility testing. The breakpoints determined were recently approved by the DIN.

P474 Activity of meropenem against Gram-negative isolates from a Polish pediatric intensive care unit – part of the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) Program, 1997–2002

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Objectives: To assess the in-vitro activity of meropenem (MEM) and eight other antibiotics against Gram-negative isolates from a pediatric intensive care unit (ICU) in Poland.
Methods: Six hundred Gram-negative isolates were obtained from clinical specimens of children hospitalized in the ICU during 1997–2002. The isolates were identified using conventional methods. Minimum inhibitory concentrations (MICs) of MEM, imipenem (IMP), piperacillin + tazobactam, cefotaxime, ceftazidime, cefepime, gentamicin (GM), tobramycin (TM) and ciprofloxacin (CIP) were determined using the NCCLS agar dilution method.
Results: The collection of Gram-negative isolates included *Escherichia coli* ($n = 90$), *Enterobacter cloacae* ($n = 122$), *Klebsiella oxytoca* ($n = 46$), *Klebsiella pneumoniae* ($n = 106$), *Serratia marcescens* ($n = 27$), *Acinetobacter baumannii* ($n = 52$), *Pseudomonas aeruginosa* ($n = 123$) and other species ($n = 34$). MEM and IMP were the most active of the agents tested with >90% of all species, with the exception of *P. aeruginosa*, susceptible at NCCLS breakpoints. MIC90 values (mg/L) of MEM remained relatively constant over the 6-year period at 0.03–0.125 for Enterobacteriaceae, one for *A. baumannii* and eight for *P. aeruginosa*. MIC90 values (mg/L) of IMP were 0.25–0.5 for Enterobacteriaceae, 1 for *A. baumannii and* 16 for *P. aeruginosa*. CIP had MIC90 values of 0.015–2 mg/L against all isolates, with the exception of *A. baumannii* (16 mg/L). Resistance to aminoglycosides (GM and TM) was a characteristic of >40% of tested isolates. The incidence of beta-lactamase-producing isolates remained at a relatively constant but high level during the 6-year period (18.5–47.7%). Incidence of AmpC production was higher than ESBL production in each year of the study.
Conclusions: MEM and IMP were the most active antibiotics tested (>90% susceptibility for all species except *P. aeruginosa*), with no observed reduction in activity over 6 years.

P475 Beta-lactamase production and susceptibility of *Capnocytophaga* to beta-lactam antibiotics

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Objectives: In recent years, the antimicrobial susceptibility spectrum of anaerobie gram-negative rods has undergone gradual change. The emergence of bacteria resistance to beta-lactam antibiotics should be noted. Beta-lactamase is the major cause of bacterial resistance to beta-lactam antibiotics. Previous studies have reported the beta-lactamase production, by 34 from 43 clinical pharyngeal isolates of *Capnocytophaga* spp. in Department of Pediatric Oncology (CHU, Rennes, France).

Methods: In this study, the beta-lactamase of 5 from the 34 strains, which belong all to *Capnocytophaga ochracea* were biochemical and genetic analyzed. These beta-lactamase positive strains resistant to first and second generation cephalosporins were markedly different in antimicrobial susceptibility to third generation cephalosporins. The crude beta-lactamase extract was prepared by sonication and triton treatment.
Results: Beta-lactamase activity was confirmed by nitrocefin test. Isoelectric focusing experiments with crude extracts achieved as a single band with an estimated pI of 5.6. Plasmid DNA isolated by alkaline lysis followed by electrophoretic analysis showed three plasmidic bands in two strains. For the three other strains, we detected no plasmid, indicating a chromosomal location of the beta-lactamase gene, without excluding a mobilizable transposon.
Conclusion: These results could explain significant divergence susceptibility to beta-lactam antibiotics, when the transposon was inserted into the chromosome or plasmid locus.
Acknowledgement: Supported by Conseil Régional de Bretagne.

P476 In vitro activity of eight oral cephalosporins against *Borrelia burgdorferi*

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Objectives: To test larger numbers of *Borrelia burgdorferi* isolates derived from different clinical and geographic sources against oral cephalosporins.
Methods: This study investigated the in vitro activity of eight oral cephalosporins, in addition to ceftriaxone and apramycin, against 17 isolates of the *B. burgdorferi* complex, including one *B. valaisiana* and one *B. bissettii* tick isolate. Minimal inhibitory concentrations (MICs) were determined by a standardized colorimetric methodology in BSK-medium after 72 h of incubation. The rank order of potency on a μg/mL basis for the substances with in vitro activity against *B. Burgdorferi* was ceftriaxone (MIC90: 0.06 μg/mL) > cefuroxime-axetil (MIC90: 0.25 μg/mL) > cefixime (MIC90: 1 μg/mL), cefdinir (MIC90: 1 μg/mL) > cefpodoxime (MIC90: 8 μg/mL) > ce-/mL) > ceftibuten (MIC90: 32 μg/mL) > cefetamet-pivoxil (MIC90: >32 μg/mL), loracarbef (MIC90: 32 μg/mL) > apramycin (MIC90: >64 μg/mL).
Conclusions: Our study demonstrates the superior in vitro effectiveness of ceftriaxone in addition to good to excellent activity on the part of the oral agents cefuroxime-axetil, cefixime, and cefdinir against *B. burgdorferi* under strictly standardized test conditions.

P477 Comparative in vitro activity of cefpodoxime with other cephalosporins against different isolates of Enterobacteriaceae

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Objectives: Cefpodoxime proxetil is a new orally administered third-generation cephalosporin. This study was performed to compare the in vitro activity of cefpodoxime (CPD) with other cephalosporins commonly used in isolates of Enterobacteriaceae.
Methods: A total of 100 strains belonging to Enterobacteriaceae (45 *E. coli*, 32 *Klebsiella*, 5 *Enterobacter*, 5 *Proteus*, 2 *Morganella*, 3 *Providencia*, 3 *Serratia* and 5 others) were collected from patients admitted to our hospital during the last two years period. Antibiotic susceptibilities were determined by standard disc diffusion procedure and the evaluation was done according to NCCLS criteria. CPD discs was provided by Sankyo Co. Japan. Tested other cephalosporins were cefepime (FEP), ceftazidime (CAZ), ceftriaxone (CRO), cefoperazone (CEP), cefaclor (CEC) and cefixime (CFM).
Results: For all isolates, the susceptibility to CPD was 71%. CPD showed a broad spectrum and potency inhibited 71, 63 and 80%, respectively, of *E. coli*, *Klebsiella* and *Proteus-Providencia-Morganella* strains. CPD was found more active than CRO, CEP, CEC and CFM. The susceptibility to CAZ and FEB were equal, for *E. coli*, *Klebsiella* and *Proteus-Providencia-Morganella* group, respectively, 73, 69 and 80%.
Conclusions: These results showed that cefpodoxime had a good in vitro activity similar to CAZ and FEB, against Enterobacteriaceae. However the overall susceptibitiy rates to cephalosporins that we determined, indicates a rising resistance problem on these antibiotics.

P478 Postmarketing surveillance study with piperacillin/tazobactam in patients with moderate or severe infections

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Objectives: piperacillin/tazobactam (P/T) is a widely used combination of the acylaminopenicilline piperacillin and the betalactamase inhibitor tazobactam. The objective of this Postmarketing Surveillance Study (PMS) conducted in 2000/2001 in Germany was to investigate the efficacy and safety of P/T in patients with moderate and severe infections.
Methods: A total of 7470 patients (mean age of 60 years) were treated with P/T. Fifty-seven percent were male, 42% female. Over 90% of the patients had moderate or severe respiratory tract infections (pneumonia), intra-abdominal infections (peritonitis, appendicitis, cholangitis), skin/soft tissue infections, other infections (urinary tract infections, FUO, sepsis) or more than one infection. Eighty-five percent of the patients had concomitant diseases (mainly cardiac disorders, diabetes mellitus, neoplasia). Thirty-one percent received a previous antibiotic treatment (mostly cephalosporines and chinolones).
Results: P/T was given 8.6 days in average. Eighty-six percent of the patients received P/T as a calculated antibiotic treatment. Seventy-six percent of patients were treated with P/T i.v. every 8 h 22% of the patients received a combination therapy (mostly aminoglycosides (12%) and chinolones (14%)). Surgery was performed in 49% of the patients (mostly in intra-abdominal and skin/soft tissue infections). Sixteen percent of patients had a change-over of therapy (in most cases to chinolones and carbapenems). A clinical response (cure or improvement) was stated in 87% of patients. An analysis of subgroups of patients with severe infections, patients treated on ICUs and mechanically ventilated patients also proved the result. The clinical cure or response rates were confirmed by an improvement of selected laboratory findings (temperature, leucocytes, CRP). In 63% of patients susceptibility data were documented. The microbiological findings of the isolated pathogens showed a high susceptibility (87%) of the most common strains. Adverse events occurred in only 7.2% of patients, only 1.8% of them were drug-related. The most common reactions were diarrhea, exanthema, hypokalaemia and allergy.
Conclusions: High response rates of P/T in the treatment of patients with moderate or severe infections were observed. P/T has an excellent safety profile. Clinical and microbiological response rates during routine treatment confirm data of previous clinical trials.

Epidemiology of resistance 1

P479 Reduced susceptibility of penicillin in Viridans Group Streptococci in the oral cavity in patients with hematological diseases

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Objectives: Viridans Group Streptococci (VGS) have become common pathogens causing septicemia in patients with hematological diseases. A reduced susceptibility to penicillin is reported (Westling, Scand J Infect Dis 2002; 34: 316–9).
Methods: Fifty patients from the Division of Hematology 2000–2002 with either newly diagnosed acute leukemia or patients in whom autologous peripher stem cell transplant was performed participated in the study. Samples were collected from saliva by mouthwash with 10 mL saline. The collection of samples was performed once a week. Aerobic and anaerobic cultures were performed. The typing of VGS was made using mitis salivarius blood agar (Difco). API STREP and API ZYM (Biomérieux, Lyon) were used for identification of VGS strains. Antibiotic susceptibility testing to penicillin for

the isolated VGS was performed with the *E*-test method (AB Biodisk, Solna, Sweden) on Mueller-Hintons medium. Isolates of VGS resistant to penicillin (MIC > 2.0 mg/L) were also tested for susceptibilities to linezolid, erythromycin, vancomycin and ciprofloxacin. Clinical data as fever and mucositis on admission were registered. Chemotherapy, ongoing antibiotic treatment, antibiotic prophylaxis, as well as previous antibiotic treatment up to 1 year before start of the study were registered.
Results: One patient was excluded because of a *Staphylococcus aureus* infection in the stem cell harvest. In 48/49 patients VGS was isolated from the oral cavity. Twelve of 48 (25%) patients had VGS strains that were resistant to penicillin (MIC > 2.0 mg/L). Eleven of the isolates were identified as *Streptococcus mitis* and one as *Streptococcus sanguis*. The patients that harboured penicillin resistant VGS (MIC > 2.0 mg/L) had more septicemias ($P = 0.04$) and more days of treatment with trimethoprim-sulphametoxazole than patients with susceptible or intermediately resistant VGS ($P = 0.04$). There were no other statistical significant differences between the two groups. Four of 12 isolates resistant to penicillin were also resistant to erythromycin (MIC > 0.5 mg/L).
Conclusions: We summarize that 25% of the patients had oral VGS resistant to penicillin that is higher than expected. This group of patients gets higher burden of antibiotic therapy due to infections after chemotherapy that might select penicillin resistant VGS strains.

P480 Epidemiology and resistance pattern of bacteremia pathogens in medical patients over a 6-year period

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Objective: To estimate difference regarding epidemiology, outcome and resistance (R) to antimicrobials of medical patients with bacteremia (B), over a 6-year period, i.e. 1997–2002.
Methods: Prospective demographic, clinical and microbiology, as well as hospital stay and outcome, data entry of medical patients with documented B. Time January 1997 to December 2002. Data entry and analysis in IBM compatible PC using EPI5-Info (CDC, 1993) programme. Sensitivity as by Kirby-Bauer, statistics by Yates corrected/2.
Results: Documented B in a total of 301 pts (M: 47.2%, F: 52.8%) mean age 69.6 years. Chronic disease present in 79.5%, and nosocomial Â in 24.5% of patients. More frequent pathogens were *E. coli* (37%) *S. aureus* (15.6%), and most common source of B was the urinary tract (42%). Main difference between first and second half of the studied period was the rise of *Klebsiella sp.* from 2.1 to 12.6% and relative rise of Enterococcal B and Candidemias. Regarding R data of Gram-pathogens, significance was most prominent to 3rd generation cephalosporins [rising from 6.9 to 20.4%, $P = 0.024$] and ciprofloxacin [9.0–19.4%, $P = 0.06$]. The annual rise of Gram + cocci percentage is noted, without, fortunately, a rise to either MRSA rates or glycopeptide R. Mean hospital stay was 13.4 days and mortality during stay was 18%, though not always directly attributed to B.
Conclusions: The constant variability of B pathogens, the appearance of less expected ones, and mainly R profile changes deem continuous surveillance and awareness, to ensure the optimal empirical antimicrobial choice based on the most recent data of the given milieux.

P481 Detection of rifampicin and izoniazid resistance in *Mycobacterium tuberculosis* strains from Samara Region (Central Russia)

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Introduction: Recently high rates of tuberculosis incidence and prevalence are observed in civilian and prisons sectors in Russia. One of the main reasons for high morbidity levels and ineffectiveness of treatment is wide spreading of drug resistant *Mycobacterium tuberculosis* strains, but accurate and comprehensive information on levels of drug resistance among strains circulating in Central Russia is unavailable.
Objective: Rifampicin and izoniazid resistance detection in TB isolates from Samara (Central Russia) civilian and prison TB hospitals and dispensaries in 2000–2002 by revealing mutations in rpoB, katG and inhA genes using Macroarray technique.
Methods: A total of 342 *M. tuberculosis* isolates were tested using Macroarray method. It is based on multiplex amplification of *rpoB*, *katG* and *inhA* genes fragments (with three pairs of biotin labeled primers) following by dot-hybridization with normal and mutant oligonucleotide probes (fragments of *rpoB*, *katG* and *inhA* genes in which mutations occur) immobilized on nylon membrane strips. Mycobacterial DNA was extracted by heating of cell suspensions following by chloroform extraction. Streptavidin-alkaline phosphatase color development system was used for visualization of results.
Results and conclusions: In total, 78.8% of isolates was determined to be resistant to one or more drugs. From those, 66.3% were resistant to rifampicin and 92.9% to izoniazid. Izoniazid resistance in the most part of isolates (88.4%) was due to the mutation in 315 codon of katG gene. The percentage of resistant mycobacteria was higher in those isolated from prisoners than from civilians (84.7 and 75.1%, respectively). From resistant isolates 165 specimens (48.2%) possessed mutations both in rpoB and katG (or inhA) genes and were determined as multidrug resistant (MDR). It proves the fact that rifampicin resistance is very often combined with izoniazid resistance and serves as indicator of MDR. Very high prevalence of MDR *M. tuberculosis* strains in Central Russia reflects general TB situation in Russian Federation and other countries of the former Soviet Union and is a serious problem for doctors and public health in general.

P482 Clinical experience of linezolid in a UK teaching hospital

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Introduction: Linezolid, an oxazolidinone antibiotic, has a unique mode of action and is effective against a broad range of resistant and sensitive gram positive infections. In order to prevent misuse of this useful agent the antibiotic committee for Tayside hospitals decided on a number of strategies to control prescribing. These included agreement on appropriate clinical situations for use of linezolid (below), all prescriptions are subject to approval by infection specialists and continual monitoring by clinical pharmacists.
Objectives: Review the clinical experience of linezolid use and compliance with our guidelines.
Method: Clinical pharmacists recorded information on all patients prescribed linezolid and ensured approval had been obtained from an infection specialist. Medical notes were reviewed retrospectively for patients prescribed linezolid since its introduction (19 months). Data were collected on organisms, previous therapy, indications, duration of therapy, clinical and microbiological outcomes and linezolid related adverse reactions.
Results: Forty courses of linezolid were prescribed, notes were reviewed for 33 of these patients. A number of patients fitted more than one criteria for use of linezolid ($n = 45$). See table.

Clinical situation	%
VRE	9
Hypersensitivity or intolerance to previous regimen	24
Clinical failure or lack of improvement	11
Poor IV access	7
Patient no longer wishing IV therapy	24
Deteriorating renal function	9
Drug interaction with rifampicin or concern about hepatotoxicity	7
Facilitation of hospital discharge by switching to oral therapy	9

There was 100% compliance with our guidelines. Seventy-six percent of prescriptions for linezolid were for indications not currently licensed in the UK. The majority of patients (27) were treated for MRSA/MRSE infections. Eleven patients had a bone or joint infection. Thirty patients had previously been treated with a glycopeptide. The mean duration of therapy was 23 days. 28/33 patients had clinical outcome documented and 26 (92%) were classified as cure or improved. 8/33 patients had a linezolid related adverse reaction, all were fully reversible.
Conclusions: Compliance with our guidelines was excellent and our control measures for prescribing have proved effective. Linezolid was used in a wide of range of clinical scenarios with good outcomes. This study represents the first

documented UK experience of the use of linezolid since its introduction in 2001.

P483 Multiple resistance is rare in community-acquired lower respiratory tract infection in the UK and Ireland

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Objective: To assess levels of multiple antimicrobial resistance in pathogens associated with community-acquired lower respiratory tract infection.
Methods: 1328 *Streptococcus pneumoniae*, 1894 *Hemophilus influenzae* and 845 *Moraxella catarrhalis* from lower respiratory specimens were collected from 20 laboratories in the UK and Ireland in the winters of 1999–2000 and 2000–2001, excluding duplicates within 2 weeks and patients in hospital more than 48 h. Isolates were centrally tested by BSAC agar dilution MIC method and categorized by BSAC breakpoints (mg/L).
Results: The table shows results for representatives of beta-lactam, macrolide, quinolone and tetracycline classes.

Antibiotic	*S. pneumoniae* % R(MIC≥), %I [MIC range]	*H. influenzae* %R (MIC≥), %I [MIC range]	*M. catarrhalis* %R(MIC≥)
penicillin (PEN)	0.6 (2), 9.9 [0.12–1]	nr	nr
amoxicillin (AMOX)	1.3 (2)	nr	nr
ampicillin (AMP)	nr	15.6 (2)	91.0 (2)
erythromycin (ERY)	12.3 (1)	3.3 (16), 95.9 [1-8]	0 (1)
ciprofloxacin (CIP)	5.3 (4), 94.7 [≤2]	0.1 (2)	0 (2)
tetracycline (TET)	8.4 (2)	3.1 (2)	0.2 (2)

S = susceptible, I = intermediate, R = resistant, nr = not reported.

No isolate was resistant to all four drug classes.
Ninety-one (6.9%) of 1328 *S. pneumoniae* were resistant to two or more drug classes, of which 12 (0.9%) were R to three and 79 (5.9%) were R to only two. Triple R were: 8 (0.6%) AMOX/TET/ERY; 3 (0.2%) CIP/TET/ERY; and 1 (0.1%) AMOX/CIP/ERY. Double R (excluding triple R) were: 70 (5.3%) TET/ERY; 6 (0.5%) CIP/ERY; 2 (0.2%) AMOX/TET; and 1 (0.1%) AMOX/ERY. Fifty-eight isolates (4.4%) were triple reduced-S: 51 (3.8%) PEN(I + R)/TET-R/ERY-R; 5 (0.4%) PEN(I + R)/CIP-R/ERY-R and 2 (0.2%) PEN(I + R)/CIP-R/TET-R. 54 (2.9%) of 1894 *H. influenzae* were resistant to two or more drug classes, of which 1 (0.1%) was R to three and 53 (2.8%) were R to only two. The only triple R was AMP/TET/ERY. Double R (excluding triple R) were: 47 (2.5%) AMP/TET; 5 (0.3%) AMP/ERY; and 1 (0.1%) TET/ERY. 845 *M. catarrhalis* were all CIP-S and ERY-S so there were no triple R. The only double R was 1 AMP/TET.
Conclusion: This study establishes an extremely low baseline level of multiple resistance in community-acquired lower respiratory tract infection in the UK and Ireland for comparison with future years.

P484 Emergence of CTX-M-15 extended spectrum beta-lactamase producing Enterobacteriaceae in Bulgaria, Romania and Turkey

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Objectives: ESBL CTX-M-15 producing Enterobacteriaceae were first identified in India and Japan in 2000. Recently CTX-M-15 was detected in Bulgaria and Poland. We screened ESBL producers from Bulgaria, Romania and Turkey to explore further dissemination of this beta-lactamase in countries of the Balkan peninsula.
Methods: ESBL producing Enterobacteriaceae isolated from clinical specimens between 1997 and 2002 were screened for the production of CTX-M-15 by the ratio of their MICs for Cefotaxime and Ceftazidime. MICs were determined by an agar dilution technique according to NCCLS guidelines. Further analysis included transfer of plasmids and isoelectric focusing of crude homogenates. To identify the *blaCTX-M-15* gene, the PCR product obtained using oligonucleotides binding to the flanking region of the gene was sequenced.
Results: Four *E. coli* strains, one each from Galati County Hospital, Galati, Romania; Hospital of Istanbul Faculty of Medicine, Istanbul, Turkey; Pediatric Hospital and ambulatory of Medical University, Sofia, Bulgaria; and one *K. pneumoniae* from Military Hospital, Sofia, Bulgaria demonstrated a MIC ratio for cefotaxime/ceftazidime equal to or above 2 indicating the production of a cefotaximase. In all strains the encoding gene was located on a conjugative plasmid. All produced an enzyme with a pI slightly above that of CTX-M-3. The *bla*-genes could be amplified with CTX-M group specific primers. Sequencing of the genes and deduction of the amino acid sequence demonstrated identity with CTX-M-15. Epidemiological typing of the *E. coli* isolates revealed no relatedness between the different isolates. However spread of plasmids may have occurred as identical plasmid fingerprints were detected.
Conclusion: CTX-M-15 producing organisms spread further in Bulgaria and emerged for the first time in Romania and Turkey.

P485 Detection of extended spectrum beta-lactamases in *Klebsiella pneumoniae*

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Extended-spectrum beta-lactamases (ESBLs) confer resistance to newer cephalosporins and penicillin with expanded-spectrum activity. ESBLs are mostly plasmid-mediated enzymes. ESBLs were first described in 1983. ESBLs can be detected by double disk synergy (DDS), modified DDS (MDDS) and *E*-test methods. In this study the presence of ESBL was investigated by DDS and MDDS methods in 100 *Klebsiella pneumoniae* strains isolated from clinical specimens such as urine (47), blood (24), wound (11) and other sites (18). The presence of ESBL was detected in 15% of isolates by DDS method and in 47% of isolates by MDDS method. *K. pneumoniae* strains were also tested for susceptibility to aztreonam, cefotaxime, ceftazidime, cefoperazone, cefoxitin and imipenem. Intermediate susceptible isolates were classified as resistant. The ratio of resistant isolates were found as 59% for aztreonam, 50% for cefotaxime, 50% for ceftazidime, 60% for cefoperazone, 12% for cefoxitin and 0% for imipenem. In-vitro susceptibilities of 47 ESBL producing *K. pneumoniae* strains to these antibiotics found as 0% for aztreonam, 4.25% for cefotaxime, 4.25% for ceftazidime, 4.25% for cefoperazone, 89.3% for cefoxitin and 100% for imipenem. Because of the high prevalence of ESBL producing strains of *K. pneumoniae* isolated from clinical specimens clinical microbiology laboratories must effectively detect and report the presence of ESBL routinely.

P486 Analysis of dihydropteroate synthase genotype of *Pneumocystis carinii* from AIDS patients with recurrent pneumonia

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Objective: Failure of sulfa or sulfone prophylaxis is associated with mutations in *Pneumocystis carinii* gene coding for dihydropteroate synthase (DHPS). We investigated whether selection of these mutations can occur within patients under drug pressure.
Methods: DHPS genotype was analyzed in patients with two separate episodes of *P. carinii* pneumonia (PCP). *P. carinii* was typed using the PCR-single-strand conformation polymorphism multilocus method.
Results: In five of seven cases with both episodes caused by the same *P. carinii* type, a switch to mutant DHPS strain from either wild type DHPS or a mixed wild type/mutant DHPS between first and second episode of PCP was observed. The two remaining patients had a mutant strain already at the first episode. All patients had received treatment or maintenance therapy with cotrimoxazole or dapsone.
Conclusion: The results suggest that *P. carinii* DHPS mutations can be selected de novo within patients under drug pressure.

P487 Increased treatment failure after 3-day courses of nitrofurantoin and trimethoprim for urinary tract infections in women

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Objectives: To assess the incidence rate and the determinants of treatment failure after antimicrobial therapy of urinary tract infections in women.

Methods: The rate and the determinants of treatment failure after antimicrobial therapy of urinary tract infections were assessed in a cohort of 16703 Dutch women who received a course (3, 5 or 7 days) of trimethoprim, nitrofurantoin or norfloxacin between January 1, 1992 through December 31, 1997 and who were between 15 and 65 years at the day of first use. A further prescription for one of these three antibiotics or for cotrimoxazole, amoxicillin, ciprofloxacin or ofloxacin, within 31 days after the end of the initial therapy was defined as an indicator for failure of the initial treatment. Determinants of treatment failure were identified 1 year prior to the start of the first dispensing.
Results: Treatment failure rate was 14.4% in patients treated with trimethoprim and nitrofurantoin and 9.6% in patients treated with norfloxacin. A multivariate analysis showed that 5- (RRnit 0.67, 95% CI 0.57–0.82, RRtri 0.82, 95% CI 0.73–0.91) and 7-day (RRnit 0.64, 95% CI 0.53–0.77, RRtri 0.85, 95% CI 0.71–1.02) trimethoprim and nitrofurantoin treatment appeared to be more effective than a 3-day treatment (reference category). Compared to a 3-day nitrofurantoin treatment (reference) a 7 days norfloxacin treatment had the lowest treatment failure rate (RR 0.42, 95% CI 0.30–0.57). Other factors increasing treatment failure rates were the age of the patient, the year of therapy and previous hospitalization. In contrast, previous use of oral contraceptives, hormonal replacement therapy, antidiabetics and oral corticosteroids did not significantly affect the treatment failure rate.
Conclusions: Most national guidelines on the therapy of uncomplicated urinary tract infections in women advise to prescribe 3-day courses of trimethoprim and/or nitrofurantoïn. However, our data indicate that for certain groups of female patients (for example, the older and recently hospitalized) a 3 days course may not be sufficient. Therefore, we suggest that the optimal antimicrobial therapy for the treatment of urinary tract infections in women is not only based on predefined standards but also on background information of the patient.

P488 Decreased invasive capacity of penicillin-resistant pneumococci

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Objective: *Streptococcus pneumoniae* is a major cause of morbidity and mortality in all age groups. In a few year penicillin nonsusceptible pneumococci have emerged worldwide as a new threat. The objective of our study was to determine the relationship between susceptibility and virulence.
Methods: We collected data regarding all strains of *S. pneumoniae* which were identified in our hospital in Madrid during a 44-month period from April 1998 to December 2001. Susceptibility patterns to penicillin were classified as intermediate (MIC 0.125 to <2.0 μg/mL) and resistant (MIC >2.0 μg/mL) as recommended by NCCLS.
Results: During the study period 229 different strains were identified. Of these, 83 (34%) were recovered from normally sterile sites (blood and cerebrospinal and pleural fluid). Diagnosis included pneumonia (69 patients), fever without obvious focus or infection (1 patients), meningitis (2), cellulites (5), spontaneous bacterial peritonitis (1), bartolinitis (1), conjunctivitis (15), otitis (16) and respiratory infections (135). Forty-four patients had some degree of recognized immunocompromised, which was due to HIV infection in 10 patients, neoplasic disease in 23 (13 hematological neoplasia), 8 chronic renal impairment, 1 esplenectomy and 12 chirrosis. The proportion of isolates that were nonsusceptible or resistant to antimicrobials was 47.3%. In sterile specimens the rate of susceptibility was 39.5% and in nonsterile specimens 27.6% ($P=0.048$). To further assess the independent value of this association, we performed logistic regression analysis with penicillin resistance as the dependent variable. Bacteriemic disease (OR 0.52 CI 95% 0.29–0.94) and mortality (OR 2.3 CI 95% 1–5.6) were independently associated with the presence of penicillin resistance.
Conclusion: Our data suggest that penicillin resistant pneumococci is less able to cause invasive infection, and the presence or resistance increase the mortality.

P489 Epidemiology of the staphylococcal bloodstream infections in a teaching university hospital in Turkey and an analysis of methicillin-resistant *Staphylococcus aureus* isolates with pulsed-field gel electrophoresis

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Objectives: To determine the characteristics of community-acquired and nosocomial staphylococcal bloodstream infections (BSIs) and to identify clonal relationship of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates by pulsed-field gel electrophoresis (PFGE).
Methods: Two hundred and fifteen patients with staphylococcal BISs were evaluated prospectively between June 1999 and June 2001. Various risk factors, severity of the infection, complications and mortality were investigated. Clonal relationship between MRSA isolates was analyzed by PFGE.
Results: Of the 215 patients, 16.3% were community-acquired BSI and 83.7% nosocomial BSI. Twenty three of 35 (65.7%) patients with community-acquired BSI and 75.6% patients with nosocomial BSI had an underlying disease. Surgical intervention, foreign body, mechanical ventilation and total parenteral nutrition, and previous antibiotic treatment were found to be important risk factors for nosocomial BSI group. Secondary BSI was found to be higher (62.9%) in community-acquired infections than nosocomial infections (27.8%). On the other hand, primary BSI was higher in nosocomial infections (72.2%) ($P>0.05$). Nosocomial staphylococcal BSIs, catheter-related infection was observed in 72.3% of patients. Five of 35 (14.3%) with community-acquired BSI and 22 (12.2%) with nosocomial BSI died because of infection. Suppurative complication rate was significantly higher in the community-acquired infections (22.9%) than nosocomial infections (5%) ($P<0.05$). *S. aureus* was the etiologic agent in all patients with community-acquired BSIs. Of nosocomial BSIs, the pathogen was *S. aureus* in 78.9% and remaining 21.1% CNS. Methicillin resistance rate was 68.9%. The analysis of 80 MRSA isolates with PFGE showed that 10 different main clones (A–J) were present. Sixty-one of 80 (76.3%) isolates were belong to A clone.
Conclusions: Staphylococcal BSIs lead to high mortality and significant complications. The source of infection is generally intravascular catheter in nosocomial primary BSIs. The rate of methicillin resistance among the nosocomial isolates is high. Majority of MRSA isolates belong to same clone. We believed that a strict use of infection control measurements will prevent MRSA spreading in our hospital.

P490 Lack of selection of monensin-resistant bacteria

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Objectives: The concern over the use of antimicrobial agents in food animal production and their potential to select for drug resistance in the microflora of these animals has prompted regulatory authorities to request studies to assess the potential for resistance to arise with consequent cross-resistance to other therapeutic agents. Monensin is a polyether ionophore administered as a feed additive to cattle and poultry to control coccidial infections or to improve growth performance. This study is intended to develop a workable method to assess the potential for monensin-resistant bacteria to arise in vitro among normally susceptible bacterial species, in comparison to two unrelated antibiotics.
Methods: Four bacterial species (*E. coli*, *E. faecalis*, *S. aureus*, *C. perfringens*) were each serially subcultured 20 times in broth medium supplemented with sublethal concentrations of monensin, nalidixic acid or tetracycline or no supplementation. Antibiotic concentrations were increased during subculture where tolerance became apparent. The susceptibility of isolates obtained at passage 1, 10 and 20 to the three antibiotics plus an additional six unrelated antibiotics (gentamicin, ampicillin, erythromycin, streptomycin, chloramphenicol, clindamycin) were determined using standard agar dilution methods.

Results: None of the four organisms exposed to monensin yielded isolates with significantly increased MIC values. In contrast, exposure to comparator antibiotics shifted nalidixic acid MICs against *E. coli* from 2 to >128 μg/mL and against *C. perfringens* from 4 to >128 μg/mL and for *E. fecalis* the tetracycline MICs shifted from 16 to 128 μg/mL. No shifts were observed among the control cultures in the absence of antibiotic. Cross-resistance to unrelated products was not observed even where decreased susceptibility emerged.
Conclusions: Resistance to monensin by mutation and selection does not occur in vitro under the experimental conditions tested in contrast to the two comparator antibiotics.

P491 Epidemiology of antimicrobial agents resistance to *Streptococcus pneumoniae* strains gained in Far East Russia: new results

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Objectives: Resistance of main respiratory pathogens to antimicrobial agents has recently become the chief problem in treating infections of lower respiratory tract all over the world. The Russian Far East is not an exception. The whole world conducts control policy over resistance to antimicrobial agents and the problem is regularly researched in Europe and Western Russia. Nevertheless, there is still lack of data concerning such wide territory as the Russia Far East completely different from western Russia in terms of living standards of urbans and rurals, age and ethnic composition. Our study is the first to determine the susceptibility patterns of various classes of antimicrobial agents against *S. pneumoniae* isolates from Vladivostok. We used such antimicrobial agents as penicillin, ceftriaxone, cefotaxime, ciprofloxacin, levofloxacin, chloramphenicol, clindamycin, and erythromycin.
Methods: We had worked with 134 isolates of *S. pneumoniae* from lab of the Vladivostok Navy Hospital. Accordingly to NCCLS standards we used disco diffusion method (DDM) and method of dilution in broth for the determining of minimal inhibitory concentrations (MICs). The lowest concentration of antibiotic giving no visible growth was determined to be the MIC.
Results: Among all the penicillin resistant isolates (23%), 84% showed intermediate resistance and 16% were highly resistant to penicillin. Of the penicillin resistant isolates, 8.4% were resistant to erythromycin, 13.8% were resistant to third generation cephalosporins, and 10% were resistant to clindamycin. There was an overall resistance of 15% for fluoroquinolones.
Conclusion: The high level of erythromycin and penicillin resistance reported emphasizes the global trend of increasing resistance. The most applied agents in our region are erythromycin, ciprofloxacin and penicillin. Control of the problem of antimicrobial resistance will require more judicious and appropriate use of antimicrobials, the development of new agents with novel targets of action, and strategies for preventing disease from occurring in the first place. In addition, the pursuit of an understanding of resistance mechanisms and pharmacodynamics as they relate to clinical outcome must be an ongoing effort, and that knowledge must be applied to the development of more effective approaches for the treatment of community-acquired pneumonia.

P492 Current state of antimicrobial resistance of *S. pneumoniae* in Russia: results of prospective multicenter study (PEHASus-I, phase 'B')

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Objectives: To determine the antimicrobial resistance of clinical strains *S. pneumoniae* isolated in adults and children.
Methods: This study was conducted in 20 cities (Chelyabinsk, Ekaterinburg, Irkutsk, Jakutsk, Jaroslavl, Kazan, Kovrov, Krasnodar, Moscow, N-Novgorod, Novosibirsk, Novokuznetsk, Saint-Petersburg, Smolensk, Stavropol, Tjumen, Tomsk, Rjazan, Upha, Voronezh) in different regions of Russia in 2001–2002. Identification of the strains was done on the basis of colony morphology, Gram stain, optochin susceptibility and bile solubility tests. Susceptibility to penicillin G (PEN), amoxicillin (AMO), amoxicillin/clavulanate (AMC), cefotaxime (CTX), cefepime (CFP), imipenem (IMP), erythromycin (ERY), azithromycin (AZI), clarithromycin (CLA), midecamycin (MID), clindamycin (CLI), telithromycin (TEL), levofloxacin (LEV), tetracycline (TET), cotrimoxazole (SXT), chloramphenicol (CHL), vancomycin (VAN) was determined by broth microdilution. Breakpoints were those of NCCLS (2002) except for TEL (≤0.5; 1; >/= 2 mg/L) and MID (≤4; >8 mg/L).
Results: The total of 581 nonduplicate clinical strains of *S. pneumoniae* were included in this study. The susceptibility testing results are presented in the Table.

	MIC Breakpoints					
Antimicrobial	S	I	R	I/R%	MIC_{90}, mg/L	MIC range, mg/L
PEN	≤0.06	0.12–1	≥2	10/0	0.125	0.004–2
AMO	≤2	4	≥8	0	0.06	0.03–2
AMC	≤2	4	≥8	0	0.06	0.03–4
CTX	≤0.5	1	≥2	1/0	0.03	0.008–1
CFP	≤0.5	1	≥2	2/0	0.06	0.008–1
IMP	≤0.12	0.25–0.5	≥1	3/0	0.06	0.008–1
ERY	≤0.25	0.5	≥1	01 Aug	0.06	0.016–2
AZI	≤0.5	1	≥2	01 Aug	0.125	0.03–4
CLA	≤0.25	0.5	≥1	01 Aug	0.06	0.016–2
MID	≤1	–	≥1	0/5	0.5	0.03–4
CLI	≤0.25	0.5	≥1	0/3	0.03	0.016–2
TEL	≤0.5	–	≥4	0	0.03	0.002–0.25
LEV	≤2	4	≥8	0	1	0.125–16
TET	≤2	4	≥8	Mrz 25	16	0.25–32
SXT	≤0.5	01 Feb	≥4	26 May	2	0.06-8
CHL	≤4	–	≥8	0/9	4	0.06–8
VAN	≤1	–	–	0	0.5	0.03–4

Conclusions: All b-lactams retained high activity against *S. pneumoniae*. High resistance to TET and SXT compromises their usage for the empirical therapy of pneumococcal infections. LEV, TEL and VAN demonstrated excellent in vitro activity against both penicillin- and macrolide-resistant strains.

P493 Evaluation of perioperative antibiotic therapy in an oncology ENT department

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Objectives: To evaluate the efficacy and the consequences on bacterial ecology of peroperative antibiotic therapy in oncological surgery.
Methods: A prospective study from March 2001 to August 2002. Ninety-eight patients undergoing initial oncological ENT surgery were included. Antibiotic therapy, instituted during induction of anesthesia (D0), comprised amoxicillin + clavulanic acid (AUG) 1 g × 3/24 h with gentamicin (GEN) 3 mg/kg/24 h. GEN was prescribed from D0 to D2 and AUG from D0 to D5. Peroperative samples of nasal and tracheal secretions (TS) were taken for bacteriological analysis. Bacteriological analysis were performed for postoperative samples taken on days 6 and 20 (TS and peritracheal pus).
Results: Peroperative: Nasal and TS samples were obtained from 96/98 patients, with TS samples alone being taken from 2/98 patients. Twenty-seven percent (26/96) of nasal samples and 60% of TS samples (59/98) were positive. Seventy-three microorganisms were isolated (duplicate organisms for same patients excluded): 22 *Staphylococcus aureus* (SAU), 10 *Haemophilus sp.* (HAE), 6 *Streptococcus pneumoniae* (PNE), 4 *Pseudomonas aeruginosa* (PSE), 23 enterobacteria (EN) (7 *E. coli*, 3 *Klebsiella sp.*, 2 *Enterobacter sp.*, 2 *Serratia sp.*, 3 *Proteus sp.*, 3 *Morganella sp.* . . .), 4 yeasts and 4 other bacteria. 45/73 (62%) were sensitive to AUG and 62/73 (86%) were sensitive to GEN, with 66/73 (90%) being sensitive to both GEN and AUG.
Postoperative: 73/98 patients provided one or more postoperative samples. At least one sample was positive for 57/73 (78%) patients. One hundred thirteen microorganisms were isolated (duplicate organisms for same patients excluded): 16 SAU, 4 HAE, 2 PNE, 5 PSE, 56 EN (16 *E. coli*, 11 *Klebsiella* sp., 3 *Enterobacter* sp., 5 *Serratia* sp., 6 *Proteus* sp., 8 *Morganella* sp., 10 *Acinetobacter* sp., 2 *Stenotrophomonas maltophilia*, 6 yeasts and 12 other bacteria. 46/113 (40%) were sensitive to AUG and 104/113 (92%) were sensitive to GEN, with 106/113 (93%) being sensitive to both GEN and AUG.
Conclusions: Combined AUG + GEN as recommended by the SFAR, for head and neck surgery, appeared to be effective against the majority of bacteria isolated preoperatively in ENT surgery patients in our hospital. Prolonged prescription of this drug combination could, however, lead to selection of flora resistant to AUG. A study of the clinical dossiers of 98 patients is now being conducted. In view of these results, the department of ENT surgery is

currently evaluating reduction of the duration of per-operative antibiotic therapy.

P494 Effect of Lactobacillus F19 on antibiotic resistant microorganisms in the intestinal microflora

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Objectives: To examine if administration of Lactobacillus F19 in conjunction with treatment with penicillin G prevents establishment of resistant strains of enterococci, enterobacteria and *Bacteroides* spp. in the intestinal tract.
Methods: Twenty subjects admitted to the hospital receiving treatment with penicillin G were recruited to the study. None of them had taken any antimicrobial drug within the 3 months preceding the study. The administration of antibiotics followed the routines at the Division of Infectious Diseases at Huddinge University Hospital. The patients were randomized into one treatment and one placebo group and received either powdered milk only or milk with added freeze-dried Lactobacillus F19. The products were given twice daily for 14 days. Stool specimens were collected before administration of antibiotic and probiotic products on day 1, on day 10 and 30 days after the start of administration. The specimens were diluted and inoculated on selective and nonselective media. Five colonies of enterococci, enterobacteria and *Bacteroides* spp. were isolated from samples of each subject on days 1, 10 and 30. The MICs of penicillin G were determined and penicillin resistant isolates were tested for MIC of clinically relevant antimicrobial agents.
Results: No major changes occurred in the normal aerobic or anaerobic microflora during the study period apart from increased numbers of enterococci on day 30 in the active group. The total numbers of resistant microrganisms remained at the same level in both groups during the study period. One strain of *E. faecium* isolated on day 10 from an individual in the active group was resistant to penicillin G, ampicillin, imipenem and ciprofloxacin. Before the administration of antimicrobial agents a number of enterobacteria revealed decreased susceptibility to ampicillin and piperacillin/tazobactam. The MIC values increased on day 10 and decreased again on day 30 in both groups. Isolates of *Bacteroides* spp. showed reduced susceptibility to ampicillin/clavulanic acid, cefoxitin, cefoperazone and clindamycin before, during and after administration of penicillin G. There were no differences between the patient groups. Two isolates from two patients in the placebo group showed reduced susceptibility to imipenem and metronidazole.
Conclusions: The probiotic preparation used in this study did not have any effect on the establishment of antibiotic resistant strains during administration of penicillin G.

P495 Serotypic analysis of *Streptococcus pneumoniae* involved in adult respiratory tract infections in France, 2000–2001

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Objectives: The objective of this study was to determine the distribution of serotypes (ST) of *S. pneumoniae* (SP) strains involved in adult respiratory tract infections and their susceptibility to antibiotics.
Methods: Six hundred seventy-five strains of SP were collected in 30 French hospitals during the winter 2000–2001. Each participating center collected over a 6-month period five consecutive strains per month from respiratory tract infections of adult patients. MICs were determined by the agar dilution method. Determination of capsular ST was performed with specific antisera (Statens Serum Institute, Copenhague) with the quellung reaction.
Results: The most prevalent ST were the following: 23F ($n=111$, 16.4%), 19 A ($n=60$, 8.9%), 19F ($n=60$, 8.9%), 14 ($n=58$, 8.6%), 9 V ($n=56$, 8.3%), 6B ($n=50$, 7.4%), 3 ($n=40$, 5.9%) and 6 A ($n=34$, 5%). Other ST had a frequency <5%. 52.4% of the strains were covered by the conjugate heptavalent vaccine and 80.6% by the 23-valent polyosidic vaccine. The susceptibility rates to penicillin (P)/erythromycin (E) were the following: all strains: 49/52.3; ST 23F: 24.3/35.1; ST 19 A: 31.7/26.7; ST 19F: 48.3/26.7; ST 14: 10.3/19; ST 9 V: 21.4/55.4; ST 6B: 22/26; ST 3: 97.5/97.5; ST 6 A: 55.9/53. No strain was resistant to telithromycin.
Conclusions: The results of this study show that two thirds of respiratory tract infections in adults were caused by 8 ST, 5 and 7 of them being, respectively, covered by heptavalent and 23-valent vaccines. The most resistant ST was the ST 14 followed by the ST 6B. The 23F ST, most frequently isolated had also a high rate of resistance to both P and E.

P496 Evaluation of drug resistance of *mycobacterium tuberculosis* complex in urinary and respiratory samples of patients with active TB disease

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Objective: Twenty-eight patients (Tisiatric Ward, San Paolo Hospital, Bari, Italy) with both urinary and pulmonary active TB disease were investigated from January to December 2002 with regard to the susceptibility to four most commonly used antibiotics for TB therapy.
Methods: Twenty-three sputum, 1 urine, 3 bronchial lavage, and 1 pleuric liquid obtained from the patients were positive for *Mycobacterium tuberculosis* (M.T.) complex. Each sample which resulted positive derived from a single patient. M.T. complex species were isolated by using liquid broth 7H9, and the method utilized for drug-susceptibility testing was the fluorescence test (MGIT 960, Becton Dickinson). Drug susceptibility has been evaluated for Streptomycin (SM), Isoniazid (INH), Rifampin (RIF), and Ethambutol (EMB).
Result: The results are summarized as follows:

	New cases	Relapses	Total patients	(%)
	21	7	28	100
Full susceptibility	11	4	15	53.57
Resistance				
Streptomycin (SM)	2	–	2	7.14
Isoniazid (INH)	3	–	3	10.7
Rifamphin (RIF)	–	–	–	0
Ethambutol (EMB)	2	–	2	7.14
INH+ RIF (MDR)	1	2	3	10.71
INH+SM+EMB	2	–	2	7.14
INH+RIF+EMB (MDR)	–	1	1	10.71

Conclusions: Our results highlight the general increase in our area of drug-resistance for *Mycobacterium tuberculosis* complex; in particular it appears to be noteworthy- the increase in drug-resistance for more than one antibiotic, when compared with most recent reports.

P497 Study of drug resistance in pulmonary tuberculosis patients in Shandong, China

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Objective: To evaluate the pattern of drug resistance among pulmonary tuberculosis patients with sputum acid-fast bacilli smear-positive.
Methods: During 1999, all sputum smear-positive patients in 30 clinics were intake with their names, ages, sex, address, symptoms, and disease history registered at the same time. Patients with 1 month or less of prior treatment were defined as new cases; those previously treated for more than 1 month were defined as retreatment cases. Their drug resistance patterns were obtained by sputum culture for acid-fast bacilli (AFB) and sensitivity testing with isoniazid (H), rifampicin (R), streptomycin (S) and ethambutol (E) in proportion method.
Results: Of the 2106 patients evaluated, 1875 (89.0%) were culture-positive. Of the 1875 patients aged 14–86 years, 1331(71.0%)were male and 544 (29.0%) were female, 404 (21.5%) were resistant to one or more drugs, 1553 (82.8%) were new cases and 322 (17.2%) were retreatment cases. Of new cases, 259 patients were resistant to one or more drugs and the primal resistance rate was 16.7% (95%CI was 14.8% to 18.6%). Of retreatment cases, 145 patients were resistant to one or more drugs and the acquired resistance rate was 45.0% (95%CI was 37.7–52.3%). Of these 1875 patients, resistance to one drug was observed in 208 patients (11.1%), to two drugs in 119 (6.3%), to three drugs in 50 (2.7%) and to four drugs in 27 (1.4%). Single drug resistance was most commonly seen with isoniazid (132 patients, 8.5%) in new cases, and streptomycin (41 patients, 12.7%) in retreatment cases.

Multidrug-resistant (MDR) cases were not common: only 2.4% of the isolates from new cases and 15.2% of those from retreatment cases were MDR.
Conclusion: Drug resistance is a major problem in the treatment of pulmonary tuberculosis. Detection of drug resistance patterns and treatment with second-line antituberculosis drugs in appropriate regimens are necessary in the treatment of failure and relapse cases in order to reduce the emergence of multidrug-resistant tuberculosis. Continued monitoring of treads in drug resistance following DOTS implementation is needed.

P498 Glycopeptide-resistant Staphylococci in Greece

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Objectives: Staphylococci with decreased susceptibility to glycopeptides have been reported worldwide. The purpose of the present study was to determine the prevalence of glycopeptide-resistant staphylococci in two different areas of Greece.
Materials: Methods: In this study, three hundred seventy *Staphylococcus* isolates (120 *S. aureus* and 250 coagulase-negative staphylococci) clinically significant, collected between January to December 2002, from hospitalized patients in seven Greek hospitals (five in the rural area of Thessaly and two in the urban area of Athens) were included. The identification to species level was performed by coagulase test and API Staph System (BioMerieux). Routine antibiotic susceptibility testing was done by the disk diffusion method. MICs to oxacillin and to glycopeptides (vancomycin and teicoplanin) was assessed by agar dilution method according to NCCLS guidelines. Isolates with decreased susceptibility to vancomycin or teicoplanin were typed using PFGE, and were also examined for the presence of *vanA, vanB, vanC1, 2, 3* genes coding for vancomycin resistance in enterococci.
Results: Among *S. aureus* isolates none was found to be intermediate or full resistant to glycopeptides. Among coagulase-negative staphylococci though 26 isolates (10.4%) (20 *S. epidermidis*, 5 *S. haemolyticus* and one *S. hominis*), all methicillin-resistant, exhibited resistance to teicoplanin (MIC Ý16mg/L). Cross resistance to vancomycin was detected in 18 isolates (MIC: 8–16 mg/L). The remaining eight isolates exhibited susceptibility to vancomycin (MICs: 3–4 mg/L). PFGE analysis revealed the presence of nine different patterns. No isolate was found to carry *van* genes.
Conclusion: *S. aureus* isolates remain susceptible to glycopeptides so far. On the other hand, coagulase-negative staphylococci express resistance to glycopeptides (10.4%). This resistance was strongly correlated with the increased consumption of teicoplanin in Greek hospitals the last years emphasizing the need for glycopeptide resistance surveillance.

P499 Common profile in three multidrug-resistant *Staphylococcus aureus* strains isolated from blood cultures from two different cities in Romania, 1997–2001

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Objectives: (a) To develop a model for the microbiological investigation of nosocomial infections with multidrug resistant *Staphylococcus aureus* (*S. aureus*), (b) To obtain preliminary data on the epidemiology of multidrug resistant *S. aureus* isolated from nosocomial infections in Romania.
Methods: (a) *S. aureus* strains were identified using the coagulase, clumping factor, Novobiocine and beta-galactosidase tests. (b) Antimicrobial Resistance Testing was performed using the NCCLS standards. (c) Phage typing was done in accordance with the Colindale IUMS Committee for Staphylococcus Typing recommendations. (d) The HARMONY protocol for PFGE molecular typing was adapted for obtaining the strains profile. We included 32 Methicillin Resistant *Staphylococcus aureus* (MRSA) strains in the molecular study.
Results: (a) 28 from the 32 MRSA strains (87.85%) showed a high Oxacilline MIC (class 3 in the Thomasz classification). (b) No strain was VRSA or VISA. (c) Only 31.25% of the strains were Pefloxacine susceptible. (d) 84.38% of the strains showed a K-R, T-R, G-R aminoglicosides susceptibility pattern (e) Only 35.20% from the 32 MRSA strains were Rifamycine susceptible. (f) Only 40.62% from the 32 strains were bacteriophage susceptible. (g) We found the same profile for three strains isolated in the 1997–2001 interval in two different cities sited in the Northern and in the Southern part of Romania (two hospitals in Iassy and one in Bucharest).
Conclusions: (a) The model used in this study enabled us to obtain a comparable phenotypic and molecular characterization of the strains. (b) We found a high proportion of multidrug resistant strains. (c) Based on the preliminary results on the molecular profile of the strains we appreciate that there is a need to go deeper in the study of the multidrug resistant MRSA in Romania, especially in big, academic hospitals.

P500 High prevalence of mupirocin resistance among coagulase-negative Staphylococci in Greece

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Objectives: In Greece, mupirocin ointment 2% (20.000 mg/L) is used for therapy of superficial skin infections such as impetigo, infected eczema and wound infections. In the present study, we investigated the incidence of mupirocin resistance in staphylococci (*S. aureus* and coagulase-negative staphylococci) isolated during 2000–2002 in Greek hospitals.
Methods: A total of 150 *S. aureus* and 200 coagulase-negative isolates (CNS), obtained sporadically from patients at three hospitals of Greece (Central, South-western and Athens) were screened for resistance to mupirocin. All isolates were tested by disk diffusion method (DD), using a 5-mg/L mupirocin disk, and by MICs determination (*E*-test). The detection of *ile-2* gene was assessed by PCR. Molecular typing of mupirocin-resistant isolates was performed by PFGE.
Results: Of the 150 *S. aureus* isolates, two isolates (1.3%) were mupirocin-resistant by DD test. In the *E*-test, these isolates expressed MICs higher than 1024 mg/L. Among CNS, mupirocin-resistance was detected by DD test in 60 isolates (30%), including 54 *S. epidermidis*, 4 *S. haemolyticus* and 2 S hominis. The majority of these isolates (42, 70%) showed high-level resistance (MICs higher than 1024 mg/L), while the remaining isolates expressed MICs ranging from 12 to 64 mg/L (low-level resistance). All high-level mupirocin resistant isolates possessed the ile-2 gene. This gene was not detected among the low-level mupirocin resistant isolates. PFGE revealed the presence of different clones for *S. aureus* and CNS.
Conclusions: Resistance to mupirocin remains low in *S. aureus* isolates in Greece. Among CNS we have found higher rates of mupirocin resistance (30%), with 21% high and 9% low-level resistance. Continued surveillance for mupirocin resistance appears prudent where institutions utilize large amounts for topical control of cutaneous or catheter-related infections

P501 Metallo-beta-lactamase positive *Pseudomonas* isolates – Clinical experience with the first VIM positive isolates in the UK

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Objectives: We conducted an investigation into the epidemiology of the first seven Verona imipenemase (VIM) positive *Pseudomonas* isolates in the United Kingdom. It was important to ascertain if those isolates had been brought into the country from areas where those isolates are more prevalent or if they had occurred spontaneously. We also focused on the infection control measures that had been put into place.
Methods: We visited the hospitals where VIM positive isolates had been isolated and conducted a case note review and interviewed the infection control teams with help of a questionnaire. We assessed risk factors such as any

recent travel abroad or contact with patients from abroad, antibiotics usage prior to isolating VIM positive isolates along with disease related data such as underlying diseases, duration of hospitalization or stay on intensive care units.
Results: A VIM bearing *Pseudomonas aeruginosa* isolate has been isolated in the United Kingdom in 2001 followed by further *P. aeruginosa* isolates from 5 patients and a *P. putida* isolate from one patient in 2002. Two patients were bacteremic with the VIM positive isolates whereas the remaining five patients were first positive from sputum. Most patients had been given beta-lactam antibiotics and quinolones before the isolate were isolated. Particularly ciprofloxacin featured as an antibiotic that had been given to most of the patients. Carbapenems had not been given in all cases. All patients had been hospitalized for greater 3 weeks receiving antibiotics during this period before VIM positive isolates had been isolated. Evidence of cross-infection based on molecular typing of isolates occurred in one hospital only involving two patients.
Conclusions: VIM positive isolates have become a reality for the UK and it seems that the numbers of isolates are increasing. The isolates seem to have evolved in the UK save those from two Gulf states patients. Antibiotic pressure seems an important factor highlighting the importance of prudent use of antimicrobials in clinical practice. It was intriguing that the isolates showed low virulence especially when causing bacteremia and one might question if the organisms might be less fit as a result of carriage of multidrug resistance elements. The importance of infection control measures has been demonstrated for all cases and strict source isolation of patients is paramount coupled with reinforcement of standard precautions to prevent cross-infection.

P502 Antibiotic-resistant patterns of 131 Shigellae isolates

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Objectives: Evaluation of antibiotic resistance patterns of Shigellae isolates.
Methods: Study area Amiralmomenin hospital, from March 1998 to March 2002.
Type of study: Descriptive, analytical, cross-sectional and retrospective. From all patients with diarrhea stool sample were collected and stool exam and stool culture were done. Antimicrobial sensitivity were performed. The antibiotic discs were: ampicillin, aamikacin, cefotaxim, ceftizoxim, ciprofloxacin, cotrimoxazol, gentamycin, nalidixicacid, nitrofuratoin, tobramycin.
Results: From 2588 diarrheal cases, 131(5/07%)were found positive for *Shigella* sp, 62(47/3%)cases were women, 69(52/7%) cases were men. The antibiotic resistance profile were: Resistant to ampicillin 127 cases (96/9%), amikacin 10(7/7%), ceftizoxim 5(3/8%), cefotaxim 3(2/3%), ciprofloxacin 2(1/5%), cotrimoxazol 110(84%), nalidixic acid 13(9/9%), nitrofuratoin 25(19/1%), tobramycin 47(35/8%), gentamycin 49(37/4%).
Discussion: Shigellae species play a major role in diarrhea in Iran and their antibiotic patterns are of importance to shigellosis control. Cotrimoxazol (84%) and ampicillin resistance (96/9%) were important finding in this study. ciprofloxacin, cefotaxim, ceftozoxim, nalidixic acid are drugs of choice for shigelosis. Sensitivity test and antibiotic resistance are needed in shigllosis control.

Molecular diagnosis and characteristics of *Staphylococcus aureus*

P503 Rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in blood cultures by gene probe hybridization assay (EVIGENE)

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Objectives: Bacteremia caused by *Staphylococcus aureus*, and the increasing prevalence of methicillin-resistant strains, are a major cause for concern worldwide. As conventional culture and antibiotic susceptibility techniques take 48 h to identify MRSA from the time a blood culture is positive, empirical use of glycopeptides is common. Use of a rapid molecular technique for the identification of MRSA from positive blood cultures would allow early and appropriate treatment decisions to be made. This study evaluated a rapid gene probe hybridization assay in comparison with PCR and conventional culture for the detection of MRSA in blood cultures.
Methods: The EVIGENE MRSA Detection Kit (Statens Serum Institut, Copenhagen, DK) uses gene probe technology to identify MRSA on the basis of the *mecA* gene, which encodes for PBP2′, in conjunction with the *nuc* thermostable nuclease gene specific for *S. aureus*. In accordance with the manufacturer's instructions, 1 mL samples from blood cultures positive for Gram-positive cocci in clusters were incubated for 3 h in Mueller–Hinton broth prior to lysis and the addition of *mecA*, *nuc* and 16S rRNA probes. Hybridization, capture and detection of probe–target complexes were carried out in a microwell strip format with results obtained in <3.5 h. EVIGENE results were compared with those obtained by mecA/femB PCR and conventional culture on ORSA (Oxacillin Resistance Screen Agar; Oxoid) plates.
Results: In a survey of 200 blood cultures positive for putative staphylococci, the EVIGENE kit identified 18 samples as MRSA-positive. Of these, 17 were also positive by PCR. The sample that was EVIGENE-positive PCR-negative was also positive by PCR and latex agglutination when isolated bacterial colonies were tested. Incubation on ORSA and subsequent latex agglutination of deep blue colonies identified 16 samples as MRSA-positive. No false-positive EVIGENE MRSA identifications arose from the detection of *mecA* and *nuc* sequences from separate isolates.
Conclusion: The EVIGENE MRSA Detection Kit is a specific, simple-to-use molecular assay that enables the identification of MRSA in blood culture samples within a working day without any of the drawbacks of PCR. Rapid identification of MRSA from blood cultures would enable timely and appropriate treatment and the reduction of empirical glycopeptide use.

P504 Clinical evaluation of a novel isothermal signal amplification assay (CytAMP™) for rapid detection of methicillin-resistant *Staphylococcus aureus*

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Objectives: To evaluate a prototype isothermal amplification assay for the rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from patient screening swabs.
Methods: The prototype CytAMP™ MRSA assay (Cytocell Ltd) was evaluated with 396 clinical isolates of bacteria and 100 sets of MRSA screening swabs. Bacteria for specificity tests were taken direct from overnight cultures grown on agar plates, while the swabs were incubated overnight at 30°C in brain heart infusion broth containing oxacillin (2 mg/L). Both were then examined for MRSA by: (i) the CytAMP assay; (ii) conventional culture on oxacillin-resistance screening agar (ORSA; Oxoid) followed by latex agglutination tests; (iii) mecA/femB PCR. The CytAMP assay is based on isothermal signal-mediated amplification of RNA technology (SMART), which generates multiple copies of an RNA signal. This signal is detected by an enzyme-linked oligosorbent assay, which generates a color signal in a 96-well microtiter plate. The CytAMP MRSA assay detects the presence of the *coa* (coagulase) and *mecA* genes, thereby simultaneously identifying the presence of *S. aureus* and methicillin/oxacillin resistance. Results are obtained in 3.5 h.
Results: Initial experiments indicated that the CytAMP limit of detection was 1E5–1E6 CFU/assay, equivalent to 1E6–1E7 CFU/mL in the overnight broth. This level of growth was obtained with an initial MRSA inoculum of 10–50 CFU. CytAMP and PCR both detected 113 MRSA among the 396 clinical isolates of bacteria. Conventional culture indicated the presence of 109 MRSA, but this result concealed significant false-positive (19) and false-negative (23) results compared with the molecular methods. Discrepant results were also observed between the culture and the molecular methods for the 100 sets of screening swabs. CytAMP and PCR were generally in agreement, but six screening broths were negative by CytAMP and positive by PCR. Five of these contained between 1E2 and 1E5 CFU per assay (below the CytAMP detection limit) and the sixth contained 1E6 CFU/assay.
Conclusions: The prototype CytAMP assay was specific for MRSA, but was slightly less sensitive than PCR. This may be advantageous in the clinical environment where PCR yields apparent false-positive results, possibly following cross-contamination or detection of dead cells. The CytAMP assay

is user-friendly for nonmolecular biologists and lends itself well to the workflow of a diagnostic laboratory, generating results for the hospital ward before lunchtime the next day.

P505 Rapid identification of staphylococci and the *mecA* and *nucA* genes from Bactec blood culture bottles by using the LightCycler System

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Objectives: To examine the feasibility for detection of *Staphylococcus aureus* and Coagulase negative staphylococci (CoNS), and identification of methicillin resistance directly in positive BACTEC blood culture bottles using the LightCycler system.
Methods: One hundred and fifty-three consecutively positive blood culture bottles (BACTEC 960 Roche Diagnostics) obtained from 74 patients were collected. Bottle contents that signaled positive were Gram stained, and inoculated onto 5% sheep blood agar; if gram-positive cocci in clusters were seen, a 1 mL aliquot for PCR was also immediately removed and stored at −70°C until tested for molecular assay. Thirteen positive bottles with microorganisms other than staphylococci were included as controls. A molecular assay based on automated DNA extraction protocol with a MagNA Pure LC instrument and real-time PCR with a Light-Cycler instrument (Roche Diagnostics) was evaluated in 153 positive blood bottles. Oligonucleotide primers and fluorescence-labeled hybridization probes, designed for amplification and sequence-specific detection of both a 408 bp fragment within the *mecA* gene and a 279 bp fragment within the *S. aureus*-specific *nucA* gene were obtained from TIB MolBiol (Berlin, Germany).
Results: Twenty-two bottles which yield MRSA strains were correctly identified by the *nucA* and *mecA* PCR assay. However, in one bottle, a mixed culture of MRSA and MSSA was found giving PCR-positive for both *nucA* and *mecA*. All the 41 bottles which yield MSSA gave positive results for the *nucA* but negative results for the *mecA* gene. The 48 bottles that yield methicillin-resistant CoNS strains were correctly identified by PCR. In the 21 bottles with MSCoNS, *nucA* PCR was negative, but in this later case, two bottles gave positive results for *mecA* gene, and were considered as false positives by PCR. The sensitivity and specificity of the *nucA* gene assay were 100%, identifying all the *S. aureus* correctly. The sensitivity and specificity of the PCR assays for detection of methicillin resistance with the *mecA* gene in bottles containing *S. aureus* and CoNS were 100 and 97.5%, respectively.
Conclusion: This is a sensitive and highly specific method to identify staphylococci in positive blood cultures, allowing discrimination between methicillin-suceptible and -resistant strains.

P506 Highly sensitive and specific LightCycler assay for the detection and differentiation of pathogenic-important *Staphylococcus* species

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Background: Staphylococci are facultative anerobe Gram-positive spherical bacteria that grow in grape-like clusters, chains or pairs. They are ubiquitously present in the human microflora, especially on skin and mucosal surfaces. Of the so far known *Staphylococcus* species, *Staphylococcus aureus*, *S. epidermidis*, *S. haemolyticus* and some other coagulase-negative staphylococci (CoNS) are of major medical interest. *S. aureus* and *S. epidermidis* cause most of the nosocomial infections in intensive care units (ICU) resulting from surgical wound infections and intravascular catheter contamination, and leading to bacteremia and endocarditis. Moreover, staphylococci are responsible for severe hospital-acquired infections such as Osteomyelitis, septic arthithis and pneumonia.
Methods: Here, we describe the development of a highly reliable, sensitive and specific LightCycler assay for research use based on hybridization probes.
Results: The test allows PCR amplification, detection and differentiation of *S. aureus* DNA and the most important CoNS DNA. Differentiation is performed by melting curve analysis of amplicons subsequent to the PCR. Melting curve analysis reveals a single peak for *S. aureus* at 62°C, which can clearly be differentiated from CoNS peaks showing up at a lower temperature (51–57°C). Reliability of the system is given by coamplification an homologues internal control (IC) within each reaction. Spiked into the lysis buffer prior to extraction, the IC acts as an extraction and inhibition control in each individual sample in order to avoid false negative results. The described LightCycler *Staphylococcus* assay gives a sensitivity of less than 10 geq/reaction as determined by the Probit analysis.
Conclusion: The assay has been shown to be a fast and highly reliable research tool to differentiate *S. aureus* from the most important CoNS. It is suitable for a wide variety of research applications.

P507 *IcaA* and *IcaD* biofilm-controlling genes in staphylococci: PCR-based detection in clinical samples of patients with septic arthritis

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Objective: Many factors of virulence, including the ability to form slime (biofilm), seem to be important in the pathogenesis of staphylococcal septic arthritis. Coexpression of *icaA* and *icaD* genes has been demonstrated necessary for biofilm formation in strains of *Staphylococcus epidermidis* and *S. aureus*. Although culture and phenotypic tests for discrimination of biofilm-forming strains are important aids, rapid molecular assays capable of detecting *icaA* or *icaD* genes might contribute with valuable and timely information for clinicians treating septic arthritis patients.
Methods: Two separate PCR-based assays were developed targeting the locus of *icaA* and *icaD* of the staphylococcus genome. The assays were assembled as duplex systems, which included the *icaA* or *icaD* product and a second product based on universal bacterial 16S rRNA primers.
Results: Thirteen synovial fluid samples from patients with staphylococcal septic arthritis (PCR-detected) were tested on both *icaA* and *icaD* systems. A scale of positive controls from a biofilm-forming *S. epidermidis* strain was used for testing the analytical sensitivity of the assays. Two out of the 13 tested strains were *icaA* and *icaD* PCR-positive, one sample was *icaA*-positive only and eight were *icaD*-positive only.
Conclusions: Our results show that the presence of *icaA* or *icaD* genes in staphylococci from this group of staphylococcal septic arthritis samples is rather low. Since *icaA* is the main gene controlling biofilm formation in staphylococci, only the two *icaA*- or *icaD*-positive strains might be able to form biofilms. The assays may be performed directly on clinical samples.

P508 Detection of the Panton-Valentine leukocidin gene in *Staphylococcus aureus* by LightCycler PCR: clinical and epidemiological aspects

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Objectives: Panton-Valentine leukocidin (PVL) is an exotoxin produced by *Staphylococcus aureus*, affecting human leukocytes and promoting necrotizng tissue reactions. Recent studies have shown PVL-producing *S. aureus* strains to cause severe necrotizng pneumonia and primary skin infections. The aim of this study was investigate the prevalence of PVL-positive *S. aureus* in a Swedish setting, and for that purpose, a rapid and simple real-time PCR for detection of the PVL gene was developed.
Methods: *S. aureus* isolated from three different groups of patients were investigated: *S. aureus* septicemia, cutaneous infections and respiratory tract infections. A real-time PCR assay utilizing the LightCycler instrument with SYBR-Green-I (Roche Diagnostics, Mannheim, Germany) was developed for detection of the PVL gene.
Results: The PVL locus was detected in 1 of 65 strains from patients with *S. aureus* septicemia. In cutaneous cultures, collected consecutively during 2 days, 1 of 43 *S. aureus* isolates was found carrying the gene. On the other hand, in cutaneous methicillin-resistant *S. aureus* (MRSA) isolates collected since 1999, 15/25 were found to be PVL-positive. All of these MRSA were community-acquired and consisted of at least four different clones, according to the pulsotype found by pulsed-field gel electrophoresis. Also, all other *S. aureus* (non-MRSA) isolates from patients with skin infections, stored at the Department of Microbiology for various reasons during this period ($n = 48$), were analyzed, whereof one was tested positive. Finally, in all *S. aureus* strains isolated from the respiratory tract found stored in the same manner at the laboratory since 1999 ($n = 56$), four strains were positive for the PVL gene; none of these were MRSA. Two of these PVL-positive isolates were from a prospective study of pneumonia etiology during 1999–2002 including 31 *S. aureus* isolates.

Conclusions: Application of the LightCycler System represents a simple, reliable, reproducible and rapid PCR for detection of PVL gene in *S. aureus*. The PVL gene was detected in isolates from patients with recurrent primary skin infections and *S. aureus* pneumonia, but PVL does not seem to be an important virulence factor in the pathogenesis of staphylococcal septicemia. Remarkably, all PVL-positive cutaneous MRSA were community acquired and comprised of at least four different clones.

P509 Development of molecular probe using RAPD and REP for diagnosis of *Staphylococcus aureus* infection

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Objectives: The objective of the research is to develop a molecular probe for the rapid identification of an important nosocomial pathogen, *Staphylococcus aureus*. One of the major causes of intensive care unit mortality and morbidity is nosocomial infection. *S. aureus* causes multitude of infectious diseases, and is also known to confer multiple drug resistance. The conventional method for identification is time consuming and laborious. In this study, application of genomics using randomly amplified polymorphic DNA (RAPD) and repetitive sequence PCR (REP) were utilized to establish DNA fingerprints of pathogen, and from the established fingerprint, a molecular band was identified to be used as a probe for the identification of the pathogen.
Methods: Fifty *S. aureus* isolates from different hospitals in Malaysia were fingerprinted by RAPD, using arbitrary OPAE primers (6, 10, 11, 14, and 15), and REP using three primers designed from three unique repetitive sequences that were distributed over the entire *S. aureus* genome. Amplifications were performed in a volume of 25 µL of PCR cocktail containing genomic DNA, one of the arbitrary or REP primers, dNTPs mix, and Taq polymerase. Amplification was performed for 35 cycles for 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C, followed by the one single extension cycle for 7 min at 72°C. The products were electrophoresed, and the molecular probes were visually identified.
Results: All isolates were confirmed as *S. aureus* by the conventional methods. Electrophoresis of amplified products using the RAPD and REP techniques revealed DNA bands with different patterns. The number of DNA fragments for an isolate ranged from 1 to 16 bands. REP-PCR produced three molecular markers, positions 500, between 1500 and 2000 bp, and slightly above 750 bp, in all *S. aureus* isolates but not in other genera studied. The band at position 500 bp was chosen to be the molecular probe because of the small size and sharpness, clarity and distinction. The markers for RAPD were 500 bp for OPAE14 and 750 bp for OPAE15. Again, the most suitable RAPD molecular probe is the 500 bp because of the size.
Conclusion: Based on the results, REP probe would be a more suitable probe to be developed as a diagnostic probe for identifying local *S. aureus* isolates. The reason for this is that the primers for REP were designed from the repetitive sequence found in *S. aureus* genome, while the RAPD primers were random primers.

P510 BioPlex® technology: application to the simultaneous differentiation and quantification of staphylococcal epidermolysins A and B

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Objectives: Staphylococcal epidermolysins A (ETA) and B (ETB) from *Staphylococcus aureus* are proteases associated with staphylococcal scalded skin syndrome and bullous impetigo of young children, which constitute a risk of epidemics in nurseries and pediatrics. Caution of suspected cases remains frequently asked. Interestingly, the BioPlex® technology (Bio-Rad) offers a rapid and quantitative assay of antigens or antibodies in a single well.
Methods: BioPlex® is supported by a simultaneous cytometry-based detection of colored beads activated by antibodies or antigens and a phycoerythrin (PE) fluorescence-generated signal. Beads, differently colored by a mix of two fluorophores/pigments, define 100 different color regions. PE fluorescence matches with the streptavidin and biotinylated antibodies or antigens. Antibodies immobilized on beads can interact with sampled antigens, and further have been recognized by the biotinylated antibodies. Association of several different beads in a single well allows multiple detections.
Results: After the covalent binding of affinity-purified rabbit polyclonal antibodies onto beads and the biotinylation of the same antibodies, the system was evaluated towards the detection of the two corresponding purified ETA and ETB from strain culture supernatants. The method offered valuable measures from 10 to 25 000 fluorescence arbitrary units corresponding to concentrations ranging from 1 to 16 000 pg/mL, respectively, with a >99% correlation factor in linear regression curves. Controls evidenced a satisfying specificity. Repeatability essentially depends on activated beads with variation <2% between 2 and 16 ng/mL, <8% between 60 and 2 ng/mL, and from 8 to 15% between 1 and 60 pg/mL. When a series of *S. aureus* strains previously typed by radial gel immunoprecipitation (IP) (26 were ETA^-/ETB^-, 24 ETA^+/ETB^-, 9 ETA^-/ETB^+, 26 ETA^-/ETB^-), correlation was obtained for 98.8% of the strains. Amounts of ETB appeared more variable than those of ETA, but supernatants must be diluted 1/10 000 and 1/50 000. If fact, one strain was revealed ETB-producer by BioPlex® whereas not by IP, and the presence of *etb* gene was further confirmed by PCR.
Conclusions: The BioPlex® assay appears as a progressive, rapid, reproducible, and easy-to-apply method for multiple assessment of antigens in a single well from a small sample, as was given as an example with staphylococcal epidermolysins, and at least as sensitive as ELISA.

P511 Highly sensitive and specific LightCycler assay for detection and differentiation of *Enterococcus faecalis* and *Enterococcus faecium* DNA

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Background: *Enterococcus faecalis* and *E. faecium* are two Gram-positive bacteria which are part of the normal human microflora of the intestine and the upper respiratoral tract. Since they are of ubiquitous spread, they are playing an important role in nosocomial infections. Especially, vancomycin-resistant *Enterococcus* (VRE) strains are of increasing threat in intensive care units (ICU) causing nosocomial bacteremia, surgical wound, or urinary tract infections. Due to the fact that *Enterococcus* ssp. contains intrinsic resistance against most known antibiotics, they are a major source of superinfections.
Methods: We have developed a highly specific and sensitive real-time PCR for use on the LightCycler instrument using hybridization probes.
Results: Intended as a life science research tool, the assay is able to detect and differentiate the two most frequent *Enterococcus* pathogens: *E. faecalis* and *E. faecium* in nucleic acid preparations from bacterial cultures, blood cultures and biological samples. Differentiation of both species is accomplished by using melting curve analysis on the LightCycler instrument subsequent to PCR amplification. Melting peaks for both *E. faecalis* and *E. faecium* are clearly distinguishable at 58 and 54°C, respectively. Moreover, the LightCycler assay offers a sensitivity corresponding to 10–20 genome equivalents/reaction (geq/PCR).Putative inhibitory effects (e.g. from interfering compounds within the sample), which might lead to false-negative results, are controlled by coamplification of an internal control (IC) within each reaction. This IC is designed to be amplified by the same primer pair but detected by an additional hybridization probe. Furthermore, the assay is fully compatible with the PCR workflow system, which further improves robustness, convenience, and reliability of the assay.
Conclusions: Our LightCycler *Enterococcus* test describes a versatile highly sensitive and specific assay to differentiate *E. faecalis* and *E. faecium*. It is perfectly suited for a wide range of research applications.

Molecular diagnosis and typing

P512 Development and validation of 14 LightCycler real-time PCR assays for the quantification or qualitative detection of common viral and bacterial infections in various specimen types

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Objectives: Quantitative measurements of microbial pathogen nucleic acids in clinical specimens may be indicative of disease severity, infectiousness, or a measure of treatment response. Our objectives were to develop eight quantitative and six qualitative assays on the Roche LightCycler™ instrument which could be applied to specimens routinely submitted to a diagnostic laboratory. Validation of the methods was performed on clinical specimens containing viruses or bacteria determined by other diagnostic methods such as culture, DFA, or PCR.
Methods: PCR primers and probes for each organism were designed based on the product size, absence of secondary structure, melting temperatures, and specificity confirmed by an NCBI database search. Positive controls (ATCC strains) for each target were cloned into the pGEM-T vector and quantified by spectophotometry. All assays were optimized for magnesium chloride and probe concentrations. PCR efficiencies were calculated for both cloned controls and clinical samples of urine, buffy coat, cervical swabs, nasopharyngeal secretions, feces, and serum, mocked with microorganism. Assays were compared to qualitative PCR and other diagnostic methods by more than one technologist. Quantitative protocols were developed and validated for CMV; HPV 16, 18, 31; RSV; HCV; *C. trachomatis*, and *Bordetella pertussis*. Qualitative assays were developed for parainfluenza 1, 2, 3; Influenza A and B; and enteroviruses. Assays used FRET probe hybridization. The HPV assays used SYBR green and the enterovirus test applied Taqman chemistry.
Results: Optimized PCR assays yielded amplification curves that were steep, well-shaped, had early crossing points of 20–30 cycles for the high copy number controls, and were evenly spaced with 10-fold dilutions of the target. The PCR efficiency for the cloned controls and the clinical specimens were similar and ranged from 1.7 to 2.1. Assays demonstrated a lower range of detection of 1–100 copies. The sensitivity of the assays ranged from 83.3 to 100%, and specificity ranged from 90 to 100%. Variations of results between technologists or diagnostic methods were minimal.
Conclusions: These protocols, with their rapid turnaround, provide new molecular tools for detecting and measuring viral and bacterial load in clinical specimens from patients with respiratory disease, transplant acquired or reactivated CMV infection, sexually transmitted human papillomavirus or chlamydia, hepatitis C virus, or enterovirus infections.

P513 Identification of viruses and bacteria by real-time nucleic acid sequence-based amplification (NASBA)

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Objectives: Nucleic acid sequence-based amplification (NASBA) is a robust RNA-amplification technology developed during the last decade and used to detect a number of pathogenic viruses and bacteria. Advantages of the method compared to RT-PCR includes that the NASBA reaction is isothermal (41°C) and, thus, does not require a thermocycler, and that DNA-free RNA is not required as a template, as the amplification is selective for RNA. This makes NASBA suitable for miniaturization into lab-on-a-chip systems. The short-time objective of this study has been to test the NASBA method on several model organisms in our laboratory: MS2 phage, HPV virus, and *Bacillus cereus*. Important aspects are sensitivity and specificity of the method compared to RT-PCR. Subsequently, we will develop NASBA detection protocols for different microorganisms related to biological warfare, and finally adapt the optimized laboratory protocols to a microchip (protoype is developed) with the ability to simultaneously detect a number of these organisms in real time.
Methods: Nucleic acids (RNA + DNA) were isolated from MS2 phage, HPV virus, and *B. cereus* using the NucliSens Basic Isolation kit (OrganonTeknika). For *B. cereus*, the lysis buffer did not lyse the cells, and the bacteria were subjected to ultrasound (while in the lysis buffer). Tenfold dilution series of RNA was amplified using the NucliSens NASBA kit (OrganonTeknika). For HPV, we used the HPV-proofer kit (NorChip AS). Amplification was performed in several different 'PCR machines'.
Results: Very good detection limits were obtained for both MS2 phage (one phage particle) and HPV virus (0.01 pmol/μL), and no signals were obtained for the negative controls (other RNA viruses and sterile water). For the MS2 sample, some free RNA may have been present in the phage lysate used (contributing to the good detection limit). For *B. cereus*, no amplification was detected. This was found to be due to the defect beacon probes (two different set of primers and probes were tested). Amplification in the SmartCycler and I-cycler yielded equally good results, while in the LightCycler it reduced the amplification efficiency, and sensitivity was seen.
Conclusions: NASBA appears to be a sensitive and robust method for detection of microorganisms. However, beacon design is critical, and several sets of primers and beacons should be tested for each organism.

P514 Identification of bacterial species using 16S rDNA signature sequences

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Objective: The objective of this study was the construction of a large set of 16S rDNA signature sequences to be used on a molecular tool platform for rapid bacterial detection and identification.
Methods: Sequencing of 16S rDNA was performed on 1324 bacterial samples representing 565 species, according to phenotypic identification. 16S rDNA sequences were fragmented in 20 or 30 mer segments. Blast searches against GenBank and Ribosomal Database Project databases were performed. The segments that displayed a perfect match or a homology with one mismatch with a single species were selected as species-specific probes. Experimental validation of the species specificity of some of these probes identified in silico was conducted by real-time PCR assays.
Results: A total of 2724 30 mer probes and 25 346 20 mer probes were found unique and specific for 325 and 648 different species represented in available databases, respectively. Twenty-five of the 30 mer probes specific for clinically important organisms such as *Enterococcus faecalis* and *Streptococcus agalactiae* were then used as TaqMan probes to check their species specificity. Up to 16 isolates of the same species for which the 30 mer probes were specific and up to 27 isolates belonging to as many as 18 other species were tested by a real-time PCR. A positive amplification signal was only obtained for isolates belonging to the species expected to be targeted by the 30 mer probe in all but one case. When only two or three mismatches were needed within the probe sequence to eliminate its species specificity, isolates of highly related species gave a delayed amplification signal that could still be differentiated from a true positive signal. Overall, TaqMan results agreed with the results expected based on the sequences of the probes and the 16S rDNA.
Conclusions: A large set of bacterial species was used to define 16S rDNA signature sequences in silico. The species specificity of a subset on these probes was successfully verified by Taqman PCR assay. These findings provide useful information to develop rapid bacterial identification kits based on 16S rDNA signature sequences.

P515 Restriction-fragment length polymorphism (RFLP)-PCR as a mechanism to discriminate between closely related organisms in a complex ecosystem

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Objectives: PCR-based methodologies for interstrain discrimination were established, based on previous observations that the major porin gene (*ompC*) sequences are highly diversified in clinical isolates of *Escherichia coli*. The aim was to study the diversity of the *E. coli* population in the stool of a patient with *E. coli* septicemia, and to characterize the infecting *E. coli*.
Methods: Ten colonies of *E. coli* from a positive blood culture and 20 colonies of *E. coli* from the stool of the same patient were analyzed. RFLP analysis was carried out on the porin genes *ompC*, *ompF*, *lamB*, *ompA*, *chuA*, and *phoE*, and

the cytoplasmic proteins gloA and mdh. In addition, PFGE was carried out, and metabolic patterns and antibiograms were established for the isolates. Sequencing of a hypervariable region of the 16 s rRNA was also carried out.
Results: RFLP analysis of the porin genes differentiated two clusters of fecal *E. coli*, one of which was identical to the strain recovered from the blood culture. The same discriminatory pattern was also obtained for gloA, whereas analysis of mdh was uninformative. The results mirrored those obtained by PFGE, indicating that the PCR-based method is equally as useful as the established technique. Sequencing of the 16s rRNA gene region did not discriminate between any of the isolates. When the isolates were analyzed by metabolic patterns and antibiograms, it was found that the strains that were not separated by molecular methods (PFGE and RFLP of porin genes) could be differentiated further. Melibiose and sucrose utilization were found to vary across the 30 isolates. In addition, the isolates exhibited MIC values for amoxicillin that ranged from 4 to 1024 mg/L. These differed further in the presence of clavulanic acid. Using these additional properties the major group of fecal organisms, identified by RFLP analysis, was further divided into three subgroups with shared metabolic properties and antibiograms. Thus, even sophisticated molecular approaches may overlook population diversity.
Conclusions: This suggests that the genes for outer membrane proteins are reliable indicators of strain diversity, but that other simpler physiological tests retain a role in discriminating between isolates. These indicated that a group of strains classified as identical by the molecular methods were clearly diverse with respect to their more general biochemical properties.

P516 Dynamics of the PCR positivity in patients with invasive meningococcal disease

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Objectives: The PCR method is widely used for the detection of the *Neisseria meningitidis* (Nm), but the dynamics of PCR positivity was not yet studied. We describe first results of our project, which is focused on the determination of the time-period after the onset of antibiotic therapy (a.o.ATB) when the DNA of Nm is still detectable in cerebrospinal fluid (CSF) and blood. The dynamics of PCR was evaluated in the relationship to the clinical course of the illness and compared to classical diagnostic methods.
Methods: We assessed the dynamics of the PCR in 19 patients hospitalized at the clinic of infectious diseases with laboratory-confirmed invasive meningococcal disease (sepsis and/or meningitis). The CSF was collected in the day of admission and control CSF samples during the hospitalization according to health status. Blood samples were collected from the 1st to the 7th day of hospitalization. The DNA was isolated by Qiagen kit, and nested PCR method was used. PCR products were detected on the 2% gel electrophoresis. We performed the PCR in 29 CSF and 62 serum samples.
Results: Csf: In the day of admission, 16 of 17 CSF samples were positive, six of them after the onset of antibiotic therapy (three of them 1 day a.o.ATB., two of them 2 days a.o.ATB, one of them 3 days a.o.ATB). In 12 patients, control lumbar punction was performed, six with positive (two of them 3 days a.o.ATB, one of them 4, 5, 6, 7 days a.o.ATB) and six with negative result (one of them 5 days a.o.ATB, three of them 6 days a.o.ATB, one of them 8, 12 days a.o.ATB). Serum: on the day of admission, 9 of 14 serum samples were positive, three of them a.o.ATB (2 days a.o.ATB). In samples collected three or more days a.o.ATB, the DNA of the Nm was not detected.
Conclusions: We conclude that till the 3rd day a.o.ATB, majority of the samples were positive. The longest period, when we confirmed etiologic agens was 7 days a.o.ATB. The evidence of presence of the pathogen and the identification of the serogroup are useful for assessment of correct diagnostic schemes, serves as an important resource for epidemiological analysis, and may result in valuable surveillance.

P517 Multilocus sequence analysis of meningococci in Scotland 20 years ago

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Objectives: Multilocus sequence analysis methods were used for the typing of meningococci in Scotland from 1982 to 1984 using a fully automated procedure. *Neisseria meningitidis* is an important cause of meningitis and bacteremia worldwide, and this study provides much needed long-term epidemiological data that are essential for understanding the meningococcus and will provide information for determining future vaccine policy.
Methods: Multilocus sequence analysis was performed which involved sequencing seven housekeeping genes (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC*, *pgm*) (MLST) and antigenically variable genes. Housekeeping alleles and sequence types were assigned by examining the MLST database (http://neisseria.org/nm/typing/mLst/). The STs were assigned to lineages with the program BURST that resolved lineages. If the lineage had previously been identified, then the previously associated ST was used for the name. The antigenically variable genes that were studied were *mynA*, *siaD*, and *porA*. The mynA indicates serogroup A, *siaD* genes provide the capsule type for serogroups B, C, Y, and W135, respectively. The *porA* gene is an outer-membrane protein (OMPs), which is used for serosubtyping. Three variable regions were looked at within porA VR1, VR2, and VR3, and they were assigned the correct variant names using the porA variable region database (http://neisseria.org/nm/typing/pora/) for VR1, VR2, and VR3 (http://www.show.scot.nhs.uk/smprl/).
Results: One hundred and fifty isolates that were present in Scotland from 1982 to 1984 were successfully characterized for MLST and for *porA*. Those isolates, which had previously not been assigned a serogroup, had characterization of the *siaD* gene performed. Of the 150 isolates, it was found that around 40% of these had a new MLST type (i.e. a combination of alleles that had previously not been found). From analysis of *porA*, it was found that the majority were of standard types. The main serogroups were B and C.
Conclusion: This has provided a unique insight into the population dynamics and evolution of meningococci causing invasive disease within Scotland. It has shown that there was a large number of STs that have not been seen before compared to the last few years, where MLST had become routine laboratory procedure within Scotland. It has also shown that there has been little change within the *porA* gene over the last 20 years, and has given much needed information towards vaccine development.

P518 Multilocus sequence typing of *Haemophilus influenzae* isolates in Scotland

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Objectives: The success of current vaccination programs against Hib is due in part to the effect of vaccination on decreasing carriage of the organism. Seven serotypes of the bacterium have been identified on the basis of capsular polysaccharides. Until the implementation of widespread vaccination programs, type b *Haemophilus influenzae* was the most common cause of meningitis in children between the age of 6 months and 2 years. Recently, there has been an increase in the number of isolates causing disease; moreover, a significant proportion of these have been nontypable. A semiautomated procedure for MLST was applied to isolates of *H. influenzae* during 2002. This allows the unambiguous characterization of all invasive isolates throughout Scotland for both typable (encapsulated) and nontypable (noncapsulated) isolates. The initial database can be found at http://haemophilus.mlst.net and presently contains the allelic profiles of over 130 isolates.
Results: A total of 42 invasive isolates were received from regional microbiology laboratories throughout Scotland. Only 59% were typable and all were serotype b strains. The remaining 41% were nontypable. All these isolates were successfully characterized using the SMPRL semiautomated MLST system.
Conclusion: Conjugate vaccines against *H. influenzae* type b have been available for a decade. There is therefore a possibility of a decrease in protection in those immunized early on in the campaign and the potential for a shift in serotype distribution. It is therefore important to determine the serotype and MLST of *H. influenzae* strains causing invasive disease in Scotland. Such data can help inform future vaccine policy and highlight at a nucleotide level the changes that are occurring under selective pressure of the *H. influenzae* population.

P519 Comparing the population structure of group B streptococcus from Israel with strains from the UK and USA by using multilocus sequence typing

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Objectives: Group B streptococcus (GBS) or *Streptococcus agalactiae* is one of the leading infectious causes of neonatal meningitis and septicemia in the developed world. However, GBS is rarely a cause of neonatal sepsis in some

parts of the world. The best estimate of neonatal GBS infection in Israel shows an incidence ranging from 0.3 to 0.6 per 1000 live births compared with an incidence ranging from 0.9 to 1.8 per 1000 live births in the UK and USA. One hypothesis for such differences may be that different strains of GBS circulate in low-incidence countries compared to high-incidence countries. We used multilocus sequence typing (MLST) to study the population structure of strains originating from Israel, UK, and USA.
Methods: A total of 103 isolates of GBS were collected from Israel, 50 isolates were collected from healthy asymptomatic pregnant mothers and 53 isolates were collected from disease-associated cases. Forty-two isolates (21 carriage isolates from healthy asymptomatic pregnant mothers and 21 invasive isolates) were included from the UK, and 22 isolates (16 invasive and 6 carriage) were included from USA. We applied MLST and capsular serotyping to all the isolates.
Results: Multilocus sequence typing revealed 39 sequence types (ST) in the Israeli collection, ST 23 was more common in the invasive group and ST 19 was more common in the carriage group (for all the collections). Sixty-five percent of all the invasive neonatal isolates from the UK and USA had ST 17, and 50% of the Israeli-invasive neonatal isolates had ST 17. Only 6% of the Israeli carriage strains were ST 17. All isolates with ST 17 complex (ST 17 and its single and double locus variants) were identified as expressing capsular serotype III.
Conclusion: ST 17 complex is the most common ST in invasive neonatal disease both in Israel, the UK, and USA. The relatively low incidence of invasive neonatal disease in Israel might be due to low maternal carriage of less virulent strains (ST 17 complex).

P520 Identification and differentiation of the *Brucella* vaccine strain Rev-1 from the natural *Brucella* strains in human disease

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Background: Brucellosis is a worldwide zoonosis. National control programs of eradication including the vaccination of sheep and goats with the strain Rev-1 (Â.melitensis biotype 1) were developed in order to eliminate the human disease. Human infection with the vaccine strain Rev-1 has been reported, but this disease cannot be identified because of the difficulty in differentiating of natural *Brucella melitensis* strains from the vaccine strain Rev-1 with conventional methods. The aim of this report is the study and differentiation of the Rev-1 vaccine strain from the natural *Brucella* strains in humans with PCR technique and digestion with restriction endonuclease.
Materials and methods: Four groups of specimens were examined: (1) 5 prototype strains of *B. abortus*, *B. melitensis* (biotypes 1, 2, 3) and Rev-1 strain; (2) 13 *B. melitensis* biotype I strains that were isolated from humans; (3) 5 serum samples from patients that were suspected for Rev-1 disease; and (4) 11 serum samples from patients without evidence of Rev-1 disease. In PCR amplification, the *Brucella omp2* gene that encodes a protein of the outer membrane, was used as DNA target. The PCR product was digested with the restriction enzyme PstI. All the strains were tested for susceptibility to streptomycin with the disk diffusion method.
Results: All *Brucella* strains and serum samples from patients gave a positive PCR result producing a 282 bp band. The digestion of the amplified DNAs revealed the fragments 238- and 44 bp in prototype strains and the fragments 282-, 238-, and 44 bp in the Rev-1 strain (group 1). In 11 out of 13 strains isolated from patients (group 2), the fragments 238- and 44 bp were revealed (natural strains), while in the other 2, the fragments 282-, 238-, and 44 bp (Rev-1) were revealed. These two strains presented resistance to streptomycin, while the other 11 strains were sensitive. The digestion of the amplified DNA revealed the fragments 282-, 238-, and 44 bp (Rev1 disease) in five serum samples of group 3 and the fragments 238- and 44 bp (natural strain-disease) in 11 serum samples of group 4.
Conclusions: The results of this study confirmed that the strain Rev-1 causes human disease in Greece. The PCR technique and the digestion with PstI facilitate the identification and differentiation of the Rev1 vaccine strain from natural *Brucella* strains. These techniques also contribute to the diagnosis and differentiation of the human brucellosis according to these agents, when the causative *Brucella* strain is not isolated.

P521 Use of broad-range bacterial 16S real-time PCR in the diagnosis of CNS infections

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Objectives: To develop a molecular method able to detect accurately and rapidly, bacterial DNA in cerebrospinal fluid (CSF) samples, and to evaluate it against cultural methods.
Methods: A broad-range real-time PCR assay, targeting the bacterial 16S rDNA gene, was designed and optimized in SmartCycler system (Cepheid, USA) using universal primers and a TaqMan probe, whose sequence was previously described (Greisen et al.). A set of ATCC strains were used as positive controls. The analytical sensitivity was evaluated with serial dilutions of *Escherichia coli* and *Staphylococcus aureus* ATCC strains spiked into negative CSF samples previously tested by the PCR assay. Nucleic acids were extracted in MagnaPureLC (Roche, Germany) after enzymatic lysis of Gram-positive bacterial cell wall, using disposable material previously decontaminated by UV (UV Crosslinker, Appligene). To prevent the amplification of contaminating DNA, a batch of negative extraction and amplification controls were included. The optimized PCR protocol was further applied to 74 CSF samples with known cultural results.
Results: The optimized PCR protocol was able to detect at least 160 CFU/mL of *S. aureus* and 200 CFU/mL of *E. coli*. No amplification was found in negative controls. With CSF samples, interpretation problems were observed in five samples with late positive results, which led to the definition of a cycle threshold value of 30 in order to differentiate true positive results from possible weak contaminations (false positive). Comparing with cultural results, we found 16 true positive results, 51 true negative results, 3 false positive results, and 4 false negative results.
Conclusions: Using as reference method, the cultural results, the real-time PCR assay developed for the detection of bacterial DNA in CSF samples, showed a sensitivity of 80%, specificity of 94.5%, a positive predictive value of 84%, and a negative predictive value of 92.7%. Together with its short turn-around time (3.5 h), the present results support the potential for the clinical application of this method if an additional identification step is included. This would provide a more accurate result, clarifying the bacterial nature (pathogenic or contamination), thus improving the predictive positive value of the test.

P522 Purified reagents enable automated DNA extraction for highly sensitive PCR in microbiology

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Objective: In the past deployment of PCR in pathogen, detection was restricted by interference due to contamination of reagents with traces of DNA from target organisms. Reagent purity on a new level was required to allow highly sensitive detection of DNA of pathogenic bacteria and fungi. Although several commercial systems for preparation of DNA from different specimen are available, no focus was directed to contamination of extremely low concentration (<10 organisms) of microbial DNA. New highly sensitive amplification and detection kits especially designed for the LightCycler Instrument require a combination of pure sample preparation reagents suited for lysis of pathogenic microorganisms and DNA isolation.
Methods: We did succeed now in developing highly purified reagents for a DNA isolation procedure applicable for a variety of specimens on the MagNA Pure LC Instrument. This level of purity was achieved by an optimized production process for prevention of contamination of microbial DNA. The performance was tested in several experiments. Specimens were pretreated for liquefying, lysis of microorganisms, and solubilization of DNA. Then, a fully automated extraction process on the MagNA Pure LC Instrument was performed. It starts with binding DNA to silica surfaced magnetic particles (MGPs) due to chaotropic salt conditions and high ionic strength. Unbound substances are removed by several washing steps. Purified DNA is eluted by low-salt buffer. The elute is ready for PCR on the LightCycler Instrument.

These elutes were utilized in subsequent analysis with the LightCycler Instrument.
Results: The kit did not show any background signal in our tests. DNA preparations were tested up to 45 cycles with highly sensitive DNA detection kits for the LightCycler Instrument for *S. aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. No single sample preparation showed positive reaction in replicate runs. The applicability to different research samples, e.g. blood culture media, urine, BAL, swab was proved in a microbiological laboratory in combination with assays on the LightCycler Instrument. Data showed excellent performance in dilution series and inoculation experiments.
Conclusion: We conclude that DNA depletion from sample pretreatment and extraction reagents enable highly sensitive and specific PCR assays for a variety of pathogens from different specimen. Such an extraction method is perfectly suited for a wide range of research applications.

P523 Multiplex PCR and reverse dot blot hybridization using amniotic fluid for the diagnosis of intrauterine infection

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Objectives: Premature birth comprises 10% of all deliveries, and is the most important cause of perinatal morbidity and mortality. Furthermore, sequelae due to premature birth, such as cerebral palsy, can be social problems. As prenatal intrauterine infection has recently been identified to be a cause of premature birth, accurate diagnosis of the amniotic infection by microorganisms became important. Nevertheless, if the causative agents of intrauterine infection such as anaerobes, fungi, and mycoplasmas are to be detected by conventional culture systems, various special media and culture conditions are required, which leads to longer detection time, markedly reduced sensitivity, and thus to the clinical uselessness. We report here the development of the technique using multiplex PCR and reverse dot blot hybridization for the diagnosis of amniotic microbial infection.
Methods: Gram-positive bacteria, Gram-negative bacteria, *Candida albicans*, *Candida* spp., *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis*, *Mobiluncus* spp., *Peptostreptococcus* spp., *Bacteroides fragilis*, and *Streptococcus agalactiae* were the selected microorganisms to be detected. Multiplex PCR was performed mainly using the primers derived from the common sequences of 16S rRNA, but specific sequences were used as primers for some species. 5′-primers were labeled with biotin. DNA probes specific to each species were designed from internal sequences of the amplified products. The DNA probes were attached to nylon membrane by dot blotter and UV irradiation and the amplified products from the multiplex PCR were subsequently hybridized. These were then made to react with streptavidin–alkaline phosphatase conjugate and NBT/BCIP for color development. Human beta-globin gene was used for the positive control.
Results: Standard strains and clinical isolates showed specific reaction patterns of reverse dot blot hybridization according to each species. The experiment with amniotic fluid samples in which the presence of microorganisms had been confirmed by species-specific PCR also showed the results that are equal to the ones obtained by the PCR.
Conclusions: The technique described here using multiplex PCR and dot blot hybridization is thought to help the diagnosis of prenatal intrauterine infection by detecting microorganisms in amniotic fluid, and thus, to possibly enable the prevention of premature birth by early treatment of intrauterine infection.

P524 New approach in epidemiology of *Streptococcus agalactiae* infection

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Objectives: *Streptococcus agalactiae* (group B streptococcus; GBS) is the leading causative agent of invasive neonatal diseases of human and mastitis of the dairy cows. Integration of IS elements, e.g. IS1548, ISSa4, IS1381 in GBS genome can inactivate the virulence properties of GBS. Recently, we performed the analysis of human GBS for the presence of different IS elements. The goal of the present study was to analyze the presence of IS elements in bovine GBS in order to compare bovine and human strains. Another subject of interest was to investigate the IS-based typing as a tool for classification and molecular epidemiology of GBS.
Methods: A total of 101 GBS strains isolated from the dairy cows were tested. These strains were analyzed by Southern hybridization and multiplex PCR. Bacterial DNA was isolated by phenol/chloroform extraction. Southern hybridization was accomplished with the 'DNA Labeling and Detection Kit', Roche (Germany). The computer techniques were used for selection of the specific primers and the analysis of DNA fragments.
Results: Serotyping of 101 bovine GBS strains revealed that most of the strains were nontypable. Multiplex PCR was developed to analyze the presence of IS elements in bovine GBS. It was found that IS861 presented in 29 strains (28.7%), IS1548 presented in 9 strains (8.9%), ISSa4 presented in 48 strains (47.5%) and IS1381 presented in 26 strains (25.7%). A total of 28 bovine GBS strains (27.7%) did not possess any of IS elements, 36 strains (35.6%) possessed one of the IS elements, 35 strains (34.7%) possessed two IS elements, and 2 strains only (1.9%) possessed three different IS elements. The strains with four different IS elements were not found. Taken together, 10 different combinations of IS elements in bovine GBS were discovered. The significant difference in the presence of IS elements in bovine strains was demonstrated in comparison with the presence of IS elements in human strains recently analyzed (Dmitriev A et al., unpublished). These data probably indicate that only a part of GBS strains can infect both animal and human.
Conclusions: These data demonstrate that combinations of IS elements in GBS genome can be used as effective criteria for molecular epidemiology. In the future, this approach can be used as an additional tool for the epidemiological control and prevention of other bacterial infections.

P525 *Clostridium perfringens* in antibiotic-associated diarrhea

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The aim of the present study was to estimate the role of *Clostridium perfringens* in antibiotic associated diarrhea (AAD). Fecal specimens of 100 patients with suspected AAD were analyzed for the presence of *C. difficile* by cultivation (CCEY), antigen detection (Triage), and detection of toxin (Triage; Oxoid). The specimens were also cultured for *C. perfringens* (P; TSC; SFP), tested for presence of *C. perfringens* enterotoxin using passive latex agglutination (pet-RPLA), and analyzed by in-house PCR for enterotoxin coding gene. *C. difficile* was recovered by culture in 24 specimens (24%). Eleven of them were also toxin A positive. *C. difficile* antigen was detected in 21 specimens (21%), 20 of them were also positive by culture. *C. perfingens* was isolated in 52 specimens (52%). Only one of the culture positive specimens (2%) was positive for enterotoxin by pet-RPLA agglutination. Enterotoxin gene was detected in nine specimens including the pet-RPLA positive specimen. In seven of them, *C. perfringens* was also isolated by culture. Only two of the specimens carrying *C. perfringens* enterotoxin gene was positive for *C. difficile* by culture. *C. perfringens*, which is part of the normal fecal flora, was isolated in 52% of fecal specimens. Only one specimen (2%) contained enterotoxin. On the other hand, enterotoxin gene was found in nine specimens. Whether the gene is only seldom expressed or the method of the toxin detection is not sensitive enough, remains to be investigated. The occurrence of the *C. perfringens* enterotoxin gene (9%) was almost similar to the occurrence of toxin A of *C. difficile*, which is known to be associated with AAD. *C. difficile* toxin A and *C. perfringens* enterotoxin gene were not simultaneously recovered in any specimen.

P526 Alternative specimen types for the detection of *Chlamydia trachomatis* infection utilizing the VIDAS PROBE *Chlamydia trachomatis* test

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Objective: The aim of this study was to evaluate the ability of the VIDAS PROBE CT Test* to detect *Chlamydia trachomatis* (CT) infections in several alternative specimen types including anal–rectal, pharyngeal, and culture media (2-SP) swabs.
Methods: The VIDAS PROBE CT test is an automated assay for the amplification and qualitative detection of *Chlamydia trachomatis* 23S rRNA. *Chlamydia trachomatis* infections are the most prevalent bacterial sexually transmitted diseases (STD) worldwide. STDs, including chlamydia, are transmitted among all sexually active people through vaginal, anal, and oral sex. Anal–rectal and pharyngeal swabs were collected from the study subjects

attending an STD clinic. Study subjects were enrolled based on several 'at risk' sexual behaviors, which included engaging in anal–rectal sex, cunnilingus, and fellatio without a condom.
Results: In anal–rectal and pharyngeal swabs, the VIDAS PROBE CT Test demonstrated excellent sensitivity (7/7 and 5/5, respectively) and specificity (60/60 and 24/24, respectively) as compared to PCR. Inhibition, as monitored by a coamplified internal control, was observed in less than 1.0% (1/156) of the specimens tested. Analytical sensitivity of the test was evaluated by serially diluting CT elementary bodies (EB) in both anal–rectal and pharyngeal swab matrices. Data indicate that the assay's analytical sensitivity in anal–rectal and pharyngeal swab matrices is comparable to that of traditional swab matrices. In addition, 63 swab specimens were collected in collection devices containing traditional chlamydial cell culture media (2-SP). The recovery rate for the VIDAS PROBE CT Test was 100% (3/3) without incidence of contamination (60/60) or inhibition (0/63).
Conclusions: The VIDAS PROBE CT Test's sensitivity and specificity for alternative specimen types suggests the feasibility for a wide range of sampling methods to complement the traditional urine, urethral, and endocervical swab samples.

*This product has not been cleared by the United States FDA and is not yet available for commercial use.

Epidemiology and surveillance of nosocomial infections

P527 Changes of microbial flora and wound colonization in burn patients

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Objective: To determine the time-related changes of microbial colonization of burn wounds and body flora of burned patients.
Methods: A prospective study was carried out in the burn unit of an university hospital, Erzurum, Turkey. Patients who were hospitalized at least 3 weeks or more over a period of 7 months (May–November 2002) were enrolled in the study. Periodic swabs were taken from burn wound, nasal, axillary, inguinal, and umblical region of the patients on admission and 7th, 14th, and 21st days of hospitalization.
Results: Fifty-one patients were included in the study. The mean age was 15 years (range 7 months−60 years) and the mean body surface area burned was 22.9% (range 5–75%). Mean hospital stay time was 36.5 days (range 21–100 days). A total of 1098 microbial isolates were detected during the study period (254 were on admission, 275 at 7th, 266 at 14th, and 303 at 21st days). Coagulase-negative staphylococci (CNS, 63.0%) and *Staphylococcus aureus* (19.7%) were the most prevalent isolates in admission cultures, followed by diphteroids (3.1%). The time-related changes of microbial flora showed a gradual decrease in the number of isolates of CNS, while there was a marked increase in the numbers of *S. aureus* and *P. aeruginosa* from admission to 21st day. At the 21st day, the most frequent organisms were *S. aureus* (37.6%), CNS (34.7%), and *P. aeruginosa* (16.2%), followed by *Enterobacter* (2.6%) and *Candida* spp. (2.3%). Unfortunately, the rates of methicillin resistance of these two staphylococci strains were increased constantly (10 to 87.7% in *S. aureus* strains, and 14.4 to 76.2% in CNS strains). Isolation of other organisms was uncommon by comparison. The study, in addition, revealed that while 35.3% of burn wounds were sterile on admission, microbial colonization reached 86.3% within the first week and 100% within 2 weeks after admission. Nasal carriage of methicillin-resistant *S. aureus* increased from 3.9 to 21.6% at the 7th day and 62.7% at the 21st day.
Conclusion: The nature of microbial wound colonization and flora changes should be taken into consideration in empirical antimicrobial therapy of burned patients.

P528 Four prevalence surveys of hospital-acquired infections in a Greek hospital

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Objectives: As is well known, the surveillance of hospital-acquired infections (HAIs) is an important component of an effective nosocomial infection control program. Prevalence surveillance is a rapid and inexpensive way to estimate the problem of HAIs. To study the problem of nosocomial infections in our hospital, four prevalence studies were made from our team during the years 1994–2000.
Methods: The first and the second studies included 288 patients each, the third 265 patients, and the fourth 273 patients (the total number of hospitalized patients at the time of the study).
Results: In the first study, a nosocomial infection was found in 20 patients, in the second in 15 patients, in the third study in 13 patients, and in the fourth in 21 patients. The overall prevalence of HAIs was 6.9, 5.2, 4.9, and 7.7% for the four studies, respectively. In the first study, among HAIs, urinary tract infections were 12 (60.0%), lower respiratory tract infections were 6 (30.0%), and surgical site infections were 2 (10.0%). In the second study, urinary tract infections were 4 (26.7%), lower respiratory tract infections were 2 (13.3%), surgical site infections were 5 (33.3%), and bloodstream infections were 4 (26.7%). In the third study, urinary tract infections were 6 (46.1%), lower respiratory tract infections were 3 (23.0%), surgical site infections were 3 (23.0%), and bloodstream infections was 1 (7.7%). In the fourth study, urinary tract infections were 7 (33.33%), lower respiratory tract infections were 5 (23.80%), upper respiratory tract infections were 4 (19.04%), surgical site infections were 3 (14.28%), and others were 2 (9.52%). The use of antibiotics among the hospitalized patients was found to be 47.2, 60.1, 58.5, and 64.1% for the four studies, respectively. The incidence of multiresistant bacteria was primarily *Enterococcus* spp. and secondarily *Pseudomonas aeruginosa*, *Enterobacter* spp., *Klebsiella pneumoniae*, *Escherirchia coli*, *Staphylococcus aureus*, *S. epidermidis*. Regarding age, the highest incidence of HAIs occurred in the third age group. Unjustified prescription of prophylactic chemotherapy was found despite the suggestions of the infection control committee.
Conclusions: Repeated prevalence surveillance is a valid way to estimate the problem of HAIs.

P529 Microbial flora identified from infected wounds at a reference tertiary care hospital in Northern Italy. Epidemiology and temporal trend in a prospective study, 1998–2002

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Objective: To assess the positive rate of cutaneous swabs of wound infections in patients (pts) followed at our University Hospital since 1998, on the ground of a number of temporal, clinical, microbiological, and antimicrobial sensitivity features of the most common isolated pathogens.
Methods: Over 1100 specimens from infected cutaneous wounds (mostly surgical in origin) were sent to our reference laboratory, with a cumulative positivity rate of 39.1%, declining from the year 1999 (39.6%) to the year 2002 (33.1%; $P < 0.001$).
Results: The majority of samples (82.4%) were identified from hospitalized the pts, compared with outpatients and the day-hospital pts. Both rate and spectrum of microbial isolation did not vary according to specimen origin, but *Staphylococcus aureus* largely prevailed (177 strains, with an incidence progressively increasing over time), followed by *Pseudomonas aeruginosa* (90 isolates), *Enterococcus faecalis* (65 strains), and *Escherichia coli* (41 isolates). *Candida glabrata* was retrieved thrice in the year 2001. In vitro antimicrobial susceptibility assays pointed out a complete sensitivity of *S. aureus* to glycopeptides (teicoplanin and vancomycin), while a progressively decreasing activity was shown by imipenem, ceftazidime, norfloxacin, netilmicin, amikacin, piperacillin-tazobactam, and ciprofloxacin, and a sensitivity lower than 50% was detected for penicillin, coamoxiclav, cefotaxime, ceftriaxone, erythromycin, clarythromycin, clindamycin, chloramphenicol, rifampicin, gentamycin, and cotrimozaxole, and methicillin resistance was even regarded in 55.7% of the isolates. When considering *P. aeruginosa*, only colistin was effective on all tested strains, followed by imipenem, ceftazidime, ciprofloxacin, piperacillin-

tazobactam, amikacin, tobramycin, and aztreonam, while piperacillin, mezlocillin, and gentamycin did not reach a 50% sensitivity rate.
Conclusion: Wound infections (mostly surgical ones) can be borne by a very large etiology spectrum, so that the resort to microbial isolation and in vitro antimicrobial assay are mandatory for an appropriate management. The elevated intrinsic and acquired antimicrobial resistance demonstrated by the two most frequent pathogens retrieved in our experience (*S. aureus* and *P. aeruginosa*), depend on hospitalization itself, large and prolonged administration of broad-spectrum antimicrobial agents as preventive and therapeutic measures, surgery, and other invasive diagnostic and therapeutic procedures.

P530 *Legionella* in Tehran hospitals' water supplies

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Objectives: Hospital-acquired legionnaires' disease has become a global public health issue. The overall risk for acquisition of *Legionella* is multifactorial, with host susceptibility (immunosuppressed patients, especially organ transplant recipients and elderly patients with chronic lung disease are at highest risk) and degree of colonization within the water supply as the most important factors. The aim of this survey was to provide information about probable *Legionella* contaminations of Tehran (capital of Iran) hospital water sources.
Methods: Fifty water samples were collected from transplant units in 4 L containers. The (hot and cold) water samples were first concentrated by passing through 0.22 μm filters and then pretreated to remove free-living legionella-like organisms. The selective and nonselective media (BCYE agar) were prepared by L-Cystein and iron salts as enrichments. The BCYE plates were inoculated with 0.1 mL of each water sample and were incubated in humidified atmosphere with 5–10% CO_2 at 37°C for up to 2 weeks along with positive control.
Results: *Legionella* species were isolated from more than 50% of water samples. *Legionella pneumophila* were predominant between the isolates.
Conclusion: The results of the investigation confirmed the existence of legionnares' disease bacterium risk in hospital transplant units.

P531 A 2-year retrospective study of nosocomial candidemia in a new Greek hospital

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Objective: Candidemia is a serious bloodstream infection, and the incidence of nosocomial fungal infection has increased in recent years. The aim of this study was to analyze the epidemiology of nosocomial candidemia in a new Greek tertiary care hospital whose activity started in 2000.
Methods: The periods (A) 1 November 2000–31 October 2001 (first year of the hospital's full activity) and (B) 1 November 2001–31 October 2002 (second year) were studied retrospectively for all episodes registered as candidemia. The blood cultures were processed and cultured automatically with Bactec 9240 system, and the identification of fungi was performed with Api 20C AUX system.
Results: During period (A), 7.346 patients were admitted to Henry Dunant Hospital and six cases of candidemia (0.81 cases/1000 admissions, or 4.4% of all bacteremia cases) were detected. The clinical strains isolated were *Candida albicans* (33.3%), *Prototheca* spp. (33.3%), *C. glabrata* (16.6%), and *C. tropicalis* (16.6%). The same strain was isolated from other sites, such as bronchial secretions, sputum, pus, urine, and intravenous catheter in all but two patients (66.6%). Four patients were hospitalized in intensive care units (66.6%) and two (44.4%) in medical wards. During period (B), 23.723 patients were admitted to the hospital and 27 cases of candidemia (1.1 case/1000 admissions, or 8.7% or all bacteremia cases) were registered. The clinical strains isolated were *C. albicans* (59.2%), *C. glabrata* (18.5%), *C. guillermondii* (11.1%), *C. tropicalis* (3.7%), *C. parapsillosis* (3.7%), and *Cryptococcus laurenti* (3.7%). The same strain was isolated from other sites, such as bronchial secretions, sputum, urine, and intravenous catheter in 18 patients (66.6%). Eight patients (29.6%) were hospitalized in intensive care units, seven (25.9%) in medical wards, six (22.2%) in renal dialysis unit, and six (22.2%) in surgical wards.
Conclusions: In our hospital, *C. albicans* is still the most frequent yeast strain isolated from blood cultures. A slight increase of nosocomial candidemia was noted during the second year of the hospital's activity, which should be attributed to the larger number of admissions, the wider spectrum of clinical syndromes admitted to our hospital during the second year, and to the expansion of certain departments such as hematology, oncology, and ICU.

P532 Nosocomial fungal infections in pediatric patients – a prospective study

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Objectives: The magnitude of the nosocomial fungal infections (NI) in children has never been deeply studied in Portugal. In order to contribute to a better knowledge about this Public Health problem, we performed a study in the pediatric Hospital D. Estefânia, aiming to determine the incidence, sites, risk factors, and to identify the predominant infecting organisms.
Methods: A prospective study was conducted in infants admitted to this hospital between January 1 1998 and March 31 2000. Standard definitions by the CDC were used. Statistical analysis was performed using the CDC software EPI info 6.0. Categorical variables were analyzed by univariate analysis with the χ^2. Significance was defined as $P < 0.05$, and Odds ratios with 95% confidence intervals were calculated. Multivariate analysis by stepwise logistic regression was used to select risk factors that best predicted the occurrence of NI.
Results: NI rate in that period was 3.0 per thousand patients (67/22.124) increasing from 1.3/1000 in 1998 to 5.3/1000 in 1999. The incidence rate was significantly higher in intensive care units (2.5%) than in all different wards (0.17%). Bloodstream (30.7%), gastrointestinal (14.9%), and urinary tract (10.4%) were the most frequent types of NI reported. *Candida albicans* (65.2%), *C. parapsilosis* (27.5%), *C. tropicalis* (5.8%), and *Saccharomyces cerevisiae* (1.5%) were the organisms associated to NI. Patients' age with NI ranged from 6 days to 13 years (median 3 years). Five risk factors for hospital-acquired infection were identified in univariate analysis: anemia (Odds ratio (OR), 6.28; 95% confidence interval (CI), 1.3–30.2), intravenous catheterization (OR, 2.96; CI, 1.4–6.5), antibiotics (OR, 2.83; CI, 1.2–6.8), parenteral nutrition (OR, 2.43; CI, 1.2–5.0), and hospitalization period for longer than 3 months (OR, 2.25; CI, 1.0–5.0). However, after adjustment by multivariate analysis, only the previous use of antibiotics stayed as the main risk factor to nosocomial fungal infection (OR, 2.86; CI, 1.0–7.9). The adjustment identified the male sex as another potential risk factor (OR, 2.33; CI, 1.1–5.0) that was not detected in univariate analysis.
Conclusions: This study confirms their importance as a heavy burden on health services. The analysis of epidemiological features of NI in the pediatric population contributed to propose surveillance measures in order to reduce their incidence and to improve a better control on risk factors.

P533 Most often isolated microorganisms from peritoneal dialysates in a public hospital, Poland

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Objectives: To evaluate the frequency of microorganisms isolated from peritoneal fluids patients with continuos ambulatory peritoneal dialysis (CAPD).
Methods: From CAPD patients with peritonitis symptoms, 96 peritoneal fluid samples were collected. The specimens were inoculated on solid media after centrifugation. Aerobic, anaerobic, and broth cultures in Bact/Alert (bioMérieux) were initiated. The pellet was Gram-stained.
Results: In the period from January to December 2002, 42 of the 94 dialysates were positive (44.6%), four of the positives were polymicrobial. Following microorganisms were isolated in monocultures: *Staphylococcus epidermidis* (20), *S. aureus* (7), *Stenotrophomonas maltophilia* (3), *Candida albicans* (2), *Klebsiella pneumoniae* (1), *Streptococcus oralis* (1), *Pseudomonas* sp. (1), *C. parapsilosis* (1). In polymicrobial infections: *Enterococcus faecalis* + *C. tropicalis* (2), *E. faecium* + *C. albicans* (1), *S. epidermidis* + *E. faecalis* + *Proteus mirabilis* (1), *Bacteroides vulgatus* + *B. uniformis* + *E. faecium*(1).
Conclusion: The most prevalent etiologic agents of peritonitis in patients with CAPD were *S. epidermidis* (47.6%) and *S. aureus* (16.7%). *Candida* isolates were responsible for six episodes of peritonitis, three of them polymicrobial. Utilization of different solid and broth media enabled good recovery of various microorganisms and appropriate antibiotic therapy based on microbiologic results.

P534 Prevalence study of hospital-acquired infection in a Greek university hospital: first announcement

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Objectives: A one-point prevalence study of hospital-acquired infections (HAI) was first carried out in a Greek University Hospital. The aim of this project was to organize a surveillance of HAI in a recently established tertiary hospital. The education of the Infection Control Team on surveillance methods was one of the major objectives of this study.
Methods: A total of 254 patients hospitalized in 15 wards were recorded in a 1-day study. All departments (surgery, internal medicine, orthopedic, obstetrics, and gynecology, neurosurgery, gastroenterology, pediatric) and intensive care units (coronary disease, neonatal, respiratory) of the University hospital were included in this surveillance study. Special attention was paid to recruit all the patients.
Results: The overall prevalence of HAI was found to be 5.9%. The most common HAI recorded involved urinary tract infections (33%), followed by lower respiratory tract infections (20%), bloodstream infections (13%), and surgical site infections (6.6%). The greatest prevalence rate was found in the department of Internal Medicine (26.6%) followed by the adult intensive care unit (20%). The duration of hospitalization, the total number of used devices, and invasive procedures were significantly correlated with HAI. Positive cultures were found in 66.6% of the cases. The most frequently isolated microorganisms were: *Enterococcus faecalis* (40%), *Pseudomonas aeruginosa* (30%), *Candida* sp. (10%), *Escherichia coli* (10%), *Proteus mirabilis* (10%). The administration of antibiotics was also recorded. The prevalence of antibiotic usage among the hospitalized patients was found to be 40%.
Conclusions: The first prevalence study of HAI in a newly established Greek University Hospital showed lower percentages of infections and antibiotic usage in comparison with similar surveillance studies, which were recently carried out in other countries or in other Greek hospitals. These results emphasize the need of rational antibiotic usage to decrease the pharmacy expenses and discourage the development of resistant microoorganisms in a new hospital environment.

P535 Two-point prevalence studies of hospital-acquired infections in the orthopedic departments of 14 Greek hospitals

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Objectives: Hospital-acquired infections (HAIs) are associated with significant morbidity and mortality. The aim of this study was to investigate the prevalence rates of HAIs, the responsible microorganisms involved, and the antibiotic use in patients admitted in orthopedic departments in 14 Greek hospitals.
Methods: Two-point prevalence studies of HAIs were carried during the years 1999 and 2000. HAIs were recorded according to the CDC definitions.
Results: Seven thousand one hundred and twenty hospitalized patients were registered during the studies. Five hundred and seventy-one (8%) of them were admitted in orthopedic departments. Out of them, 256 (44.8%) were men. Their median age was 64 (range: 1–95) years. During the study, 56/571 (9.8%) cases of HAI were identified. Thirty out of 56 patients (53.6%) had a surgical site infection (SSI), 20 (35.7%) had an urinary tract infection (UTI), and seven (12.5%) had a lower respiratory tract infection (LRTI). Three hundred and ninety-seven (69.5%) patients had a recent operation. In 45/397 (11.3%), a HAI was registered. Among the 174 patients who did not have a surgical procedure, in 11 (6.3%), a HAI was identified ($P = 0.068$). However, the type of surgical wound was one of the main risk factors for the presence of HAI. Thirty-one HAIs out of 357 (8.7%) patients with clean or clean–contaminated operation were recorded, and 13/40 (32.5%) patients with contaminated or dirty operation had a HAI ($P < 0.0001$). Responsible microorganism was found in 28 out of 56 (50.0%) cases, and 34 strains were isolated. The majority of them were Gram-negative bacteria (70.6%). The most frequently isolated microorganisms were: *P. aeruginosa* (20.6%), *Staphylococcus aureus* (17.6%), *Acinetobacter* spp. (17.6%), *Escherichia coli* (11.8%), and *Enterococcus faecalis* (11.8%). The use of antibiotics and the purpose of administration were also recorded. The prevalence of antibiotic use for any reason was 74.9%. Between them, prophylactic therapy was administered more frequently (72.3%) than the empirical (20.3%) or rational (7.4%) therapy.
Conclusion: Our data suggested that prevalence of HAIs and SSIs in orthopedic departments in 14 Greek hospitals was 9.8 and 4.5%, respectively. In the patients with HAI, Gram-negative microorganisms were the most frequently isolated pathogens. More than half (54.2%) of the hospitalized patients were on antibiotic therapy for prophylaxis.

P536 Prevalence of hospital-acquired infections in surgical patients in Greek hospitals – Results from two nationwide prevalence surveys

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Objective: The purpose of the present study was to estimate the prevalence of hospital-acquired infections (HAIs) and surgical site infections (SSIs) in operated patients in Greek hospitals.
Methods: Data for this study were derived from the database created after two nationwide prevalence studies (1999 and 2000). The Greek Infection Control Network designed and coordinated the two-point prevalence surveys.
Results: Among the 1037 patients who had at least one operation in the 1999 survey, 129 patients developed 148 (14.27%) HAIs. A total of 1093 operations were registered and 49 SSIs (4.5%) were found. Among the 868 operated patients in the 2000 survey, 82 patients developed 88 (10.13%) HAIs. A total of 902 operations were registered and 38 SSIs were detected (4.2%). SSIs were found to be 33.1% of all HAIs in 1999 and 43.2% of all HAIs in 2000. Respiratory tract infection represented 22.9 and 19.3%, urinary tract infections 18.2 and 17.0%, and bloodstream infections for 11.5 and 9.0%, between all HAIs for 1999 and 2000, respectively. The median length of time elapsed from admission to operation for patients without HAI was 1 day, and was significantly different from the patients who developed HAI (median 3.0–3.5 days). The median time (length of stay) from operation to discharge for patients without HAI was 7–8 days, and was significantly different in patients who developed HAI (26.5–28 days). The day of the study, 72.4–77.8% of the operated patients was under antibiotic therapy. The median duration of the prophylactic antibiotic use in surgical patients was 4–5 days.
Conclusion: The rates of HAIs and SSIs in operated patients have been, for the first time, estimated in Greek hospitals. Rates seem to be high, but a sufficient number of operations must be registered and analyzed for each operative procedure category in order to create benchmarks and compare rates between hospitals. Length of stay in the hospital as well as the duration of the prophylactic administration of antibiotics in these patients was found to be unacceptably high. National plan for the surveillance and control of HAIs must be developed in Greece in order to estimate (thoroughly) the situation and try to implement evidence-based infection control policies in our country.

P537 Infections in a general hospital delivery department in a country with limited resources

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Objectives: There were a few data about the frequency of infections in delivery departments in which new born children were born on time, in good health and general conditions, and were only shortly hospitalized. Data that have been announced in developed countries show the low incidence between 0.3 and 3.1/100 new-born children. The incidence rates were much higher in neonatology ICUs. There were almost no data from the countries under limited resources. Health institutions in Republic of Serbia were under serious lack of material and financial resources during the most recent years. Prevalence studies of hospital infections in health institutions during 1999, that included 678 new born children, show the high prevalence (14.6%) of hospital infections. Taking in account that the ordinary delivery

department and ICU were not considered separately for the explanation of such high rate of hospital infections, there was a need for special investigation.
Methods: During 9 months, in delivery department with 42 baby beds, 3 delivery boxes, and 1–11 child births per day, the constant, radical, round-the-clock surveillance was implemented. The data about the new-born children were collected during the hospitalization as well as 7 days after the relieve. During the surveillance, there were in use CDC (USA) definitions and criteria in form of handbook adapted to domestic health institutions. The incidence rate was calculated on 100 new born healthy children.
Results: In the period from January 01–September 30 2002, 1169 new-born children were under constant, radical, round-the-clock epidemiology surveillance. Forty-eight hospital infections were registered (incidence rate was 4.1%). Infections were registered during each month of the whole period, but with different incidence rate. The highest incidence rate was in April (6.8%), and the lowest in June (0.7%). After the highest incidence rate in April, special controlled general measures for prevention of hospital infection were implemented. According to the anatomic localization, the most frequent were infection of umbilicus (18) and skin, eye sight, and septicemia (10). Etiology diagnosis was established in 45% of the clinical demonstrated infections. The most usual cause was *Staphylococcus aureus*.
Conclusion: Infection in ordinary delivery departments under the limited resources was not a big problem if the general measures for its prevention were strictly implemented.

P538 A microbiological and epidemiological survey of cutaneous swabs from inpatients, submitted for culture in a 5-year period. Hospital- versus community-acquired infection

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Objective: To assess the epidemiological and bacteriological profile of cutaneous swabs performed in hospitalized patients (pts).
Methods: All skin swabs, purulent material, and other swabs performed from different body sites due to diagnostic or surveillance purposes were prospectively evaluated since 1998, with special attention focused on their community-acquired origin (outpatients, day-hospital pts, material obtained within 72 h of hospitalization), versus nosocomial-acquired one (inpatients admitted for >72 h).
Results: Of the 5381 swabs, 26.8% were from pts hospitalized since >3 days, but cumulative positivity rate did not prove different versus that of community samples, also considering temporal trend, nosocomial infection accounted for 32% of episodes, with the greatest frequency in the year 2001 (36.6%) versus 2000 (28.2%), while community-acquired rate was 32.9% (35.7% in 1999 vs. 30.4% in 2000). *S. aureus* was the most common organism, with a higher incidence retrieved in community samples (56.2%; $P < 0.01$), followed by *P. aeruginosa* and *E. coli*, whose origin was linked to the hospital environment (27.9 and 10.7%, respectively; $P < 0.02$ and $P < 0.04$, respectively), while no difference was found in the distribution of *E. faecalis* according to the pts location. In vitro susceptibility testing showed significant differences between nosocomial isolates versus community ones. All *S. aureus* strains were completely sensitive to glycopeptides, followed by cotrimoxazole and netilmicin (>90%), and cefotaxime, ceftriaxon, coamoxiclav, clindamycin, and chloramphenicol (>75%); methicillin-resistance was 45.6% for hospital strains versus 19.8% for community isolates ($P < 0.001$). *P. aeruginosa* was completely susceptible to colistin, followed by a >90% sensitivity for ceftazidime and imipenem, and a >60% rate for piperacillin-tazobactam, aztreonam, ciprofloxacin, and tobramycin, although nosocomial origin was borne by greater resistance levels ($P < 0.05$–0001) against ureidopenicillins, ceftazidime, aztreonam, tobramycin, and amikacin.
Conclusion: The resort to skin swabs (especially for surveillance purposes) becomes increasingly frequent so that laboratories process these specimens with growing frequency: from 902 samples in 1998 to >1500 in 2000–2002; $P < 0.001$). As a result, interpretation becomes difficult for clinicians who have to distinguish colonization from invasive infection in the compromised pts. Both etiology and antibiotic sensitivity are notably influenced by the environment and the selective pressure exerted on colonizing or infecting organisms.

P539 Bacteriological monitoring of purulent and biopsy material from infection of skin and soft tissues at a university hospital in Bologna, Italy: a prospective surveillance study

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Objective: To assess the positivity rate of cutaneous swabs of wound infections in patients (pts), followed at our University Hospital since 1998, on the ground of a number of temporal, clinical, microbiological, and antimicrobial sensitivity features of the most common isolated pathogens.
Methods: Over 1100 specimens from infected cutaneous wounds (mostly surgical in origin) were sent to our reference laboratory with a cumulative positivity rate of 39.1%, declining from the year 1999 (39.6%) to the year 2002 (33.1%; $P < 0.001$).
Results: The majority of samples (82.4%) were identified from hospitalized pts, compared with outpatients and day-hospital pts. Both rate and spectrum of microbial isolation did not vary according to the specimen origin, but *Staphylococcus aureus* largely prevailed (177 strains, with an incidence progressively increasing over time), followed by *Pseudomonas aeruginosa* (90 isolates), *Enterococcus faecalis* (65 strains), and *Escherichia coli* (41 isolates). *Candida glabrata* was retrieved thrice in the year 2001. In vitro antimicrobial susceptibility assays pointed out a complete sensitivity of *S. aureus* to glycopeptides (teicoplanin and vancomycin), while a progressively decreasing activity was shown by imipenem, ceftazidime, norfloxacin, netilmicin, amikacin, piperacillin-tazobactam, and ciprofloxacin, and a sensitivity lower than 50% was detected for penicillin, coamoxiclav, cefotaxime, ceftriaxone, erythromycin, clarythromycin, clindamycin, chloramphenicol, rifampicin, gentamycin, and cotrimozaxole, and methicillin resistance regarded even 55.7% of isolates. When considering *P. aeruginosa*, only colistin was effective on all tested strains, followed by imipenem, ceftazidime, ciprofloxacin, piperacillin-tazobactam, amikacin, tobramycin, and aztreonam, while piperacillin, mezlocillin, and gentamycin did not reach a 50% sensitivity rate.
Conclusion: Wound infections (mostly surgical ones) can be borne by a very large etiology spectrum so that the resort to microbial isolation and in vitro antimicrobial assay are mandatory for an appropriate management. The elevated intrinsic and acquired antimicrobial resistance demonstrated by the two most frequent pathogens retrieved in our experience (*S. aureus* and *P. aeruginosa*), depend on hospitalization itself, large and prolonged administration of broad-spectrum antimicrobial agents as preventive and therapeutic measures, surgery, and other invasive diagnostic and therapeutic procedures.

P540 Nosocomial infections surveillance based on microbiology laboratory

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Objective: Surveillance of the temporal trend of incidence and prevalence of the microorganisms in cultured strains, selected among those at high-risk of nosocomial infection (NI) (from ICU, Hemodialysis, neonatal ICU, etc.), to observe abnormal situations (e.g. risk of NI clusters, 'alert' microorganisms, 'alert' events).
Materials and methods: From June 2001, we recorded and processed with Excel the results of the microbiological cultures (performed in the department of intensive care, neonatal intensive care, hemodialysis, infectious diseases, respiratory diseases) to obtain the following information: (1) number of microbiological samples; (2) average (SD)of samples for patients; (3) total number of positive and negative samples; (4) number of samples for each type of material with percentage of positivity; (5) comparison between positive blood cultures from central venous catheter (CVC) and peripheral vein (to diagnose catheter-related bloodstream infections).
Results: The monthly process permitted to follow the frequency of microorganisms monitoring the positive strains from June 2001 till now. In ICU, the mostly found microorganism was *P. aeruginosa* followed by *Candida* spp., while in hemodialysis, *S. aureus* and coagulase-negative followed by *Candida*.

Conclusions: Analysis of laboratory data permitted the developing of more suitable measures to decrease the incidence of infections (e.g. asepsis of the wards, increased attention to the right nursing: hand washing, 'good practice' standards, guidelines adherence).
Future aims:
1. Beginning the analysis in others wards;
2. going on with monthly process and also with corrective measures;
3. correctly diagnosing the NI cases; and
4. continuing the training of health professionals.

P541 Post-intervention frequency of bacteriuria, pyuria and bacteremia in outpatients who have undergone cystourethroscopy

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Objectives: Urinary tract infections are the most common nosocomial infections. About 80% of nosocomial urinary tract infections are associated with the use of urethral catheters. Another 5–10% occur after genitourinary manipulation such as cystourethroscopy. The necessity of antimicrobial prophylaxis prior to cystourethroscopy is controversial. The aim of this study was to determine the rate of bacteriuria, pyuria, and bacteremia in outpatients who underwent cystourethroscopy and was not administrated antimicrobial prophylaxis prior to intervention.
Methods: Seventy-one patients who underwent cystourethroscopy for various indications and did not have bacteriuria were included. In the study, patients were not given antimicrobial prophylaxis. A midstream urine sample was taken before and 48 h after the procedure. Blood culture was taken 1 hour after cystourethroscopy. Patients were questioned for newly developed symptoms 48 h after cystourethroscopy. Blood cultures were taken again from patients who presented with fever.
Results: Six patients (8%) developed significant bacteriuria: four of them were asymptomatic and six of the patients (8%) developed pyuria without significant bacteriuria. Bacteremia was not determined in any of the patients. The association between presence of pyuria prior to procedure and development of significant bacteriuria after the procedure was significant ($P < 0.05$).
Conclusion: In our study, the rate of occurrence of significant bacteriuria after cystourethroscopy was 8%, and bacteremia did not develop. Thus, we conclude that cystourethroscopy is safe and a well-tolerated procedure. The microscopic evaluation of the urine should be carried out even if the patients do not have symptoms after the procedure, as asymptomatic bacteriuria may develop after cystourethroscopy. The presence of pyuria prior to the intervention is a risk factor, and the patients who have pyuria should be followed up due to the fact that significant bacteriuria may develop in these patients. We conclude that administrating prophylactic antibiotics for patients who have sterile urine prior to cystourehtroscopy is unnecessary.

P542 Contribution of VIGI@ct® software to nosocomial infection control in a 920-bed French hospital

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Objectives: A 6-year retrospective study was performed to analyze the contribution on the Infection Control Program of VIGI@ct (bioMérieux) epidemiology software to detect and alert on suspected nosocomial infections (NI) and multidrug-resistant bacteria (MDR).
Materials and methods: In the first period (P1: 1997–99), administrative and specimen-related data were collected, entered manually and analyzed using EpiInfo (TM; CDC, Atlanta). The suspected NI were detected manually on a monthly basis. In the second period (P2: 2000–2002, study ongoing for 2002), VIGI@ct, connected to both the Laboratory Information System and Microbiology Automated Systems, was used to gather all the data automatically. In case of suspected NI or MDR isolates, a real-time alert with a questionnaire is printed out and sent to both the infection control team (ICT) and the clinician to confirm the infection, and add any relevant information.
Results: In P1, the time-consuming manual data entry only enabled us to perform limited data analysis (i.e. incidence rate (IR) on presumptive NI, evolution of the susceptibility to antibiotics of the most frequently isolated bacteria). In P2, data from the questionnaires enabled us to calculate and analyze iatrogenic and endogenous confirmed NI IR, acquired versus imported methicillin-resistant *S. aureus* (MRSA) IR. Information such as the bacteriemia portal of entry and attributable deaths were also analyzed. Documented evolution of the susceptibility to antibiotics of the most frequently isolated bacteria were given to the clinicians. On analyzing the data, we noted a correlation between the 4% decrease of MRSA and the 15% decrease of the vancomycin hospital daily dose. Evaluation of the infection protocol's efficiency was performed to implement better protocols to control the spread of infection (e.g. 23% decrease in urinary tract infections due to catheter after 6 months) (Table 1).

Table 1

	1997	1998	1999	2000	2001	2002*
No. of inpatients	34643	35773	35185	34121	33675	27327
Suspected NIIR	3.23	4.24	4.64	–	–	–
Confirmed NIIR	–	–	–	2.69	2.76	2.93
Iatrogenic	–	–	–	1.52	1.49	1.53
Endogenous	–	–	–	1.17	1.27	1.40
Acquired versus Imported MRSAIR	–	–	–	0.27/0.73	0.20/0.91	0.22/0.96

*11-month data

Conclusion: The VIGI@ct software helps us provide clinicians and the ICT with real-time and relevant information on NI and MDR. The rapid and efficient feed-back from clinicians and the ICT enables us to improve the analysis of infection control data, and help the ICT act rapidly to increase the efficiency of the infection control program.

Fungal infections therapy

P543 Diagnosing and monitoring of invasive aspergillosis during antifungal therapy by polymerase chain reaction: an experimental study in mice

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Objectives: Invasive aspergillosis is one of the commonest causes of death due to infection in neutropenic cancer patients and recipients of allogenic stem cell transplants. Early diagnosis of infection is important as early treatment with antifungal drugs may increase the patient survival. Recently, we evaluated twice the value of weekly screening for circulating fungal DNA using polymerase chain reaction (PCR) of whole blood samples. The results indicate the usefulness when screening for *Aspergillus* in patients at risk. Hence, positive results became negative shortly after commencement of antifungal treatment, and did not correlate with underlying invasive fungal infections. In this study, we evaluated the value of PCR for diagnosing and monitoring of invasive aspergillosis during amphotericin B therapy.
Methods: Thirty-six female BALB/c mice were infected with *Aspergillus* spp. Mice were treated either with amphotericin B or glucose for 10 days. For diagnosing and monitoring PCR, microscopy and culture of tissues and blood were performed. The PCR results of treated animals were compared to those of untreated animals. DNA extraction was performed using recombinant lyticase, the genes of the 18S rRNA were amplified with PCR using specific primers, and amplicons were detected by PCR-ELISA.
Results: In the group of amphotericin B application, 27 (64.3%) tissue samples were positive by PCR, 32 (76.1%) by microscopic examination, and 20 (47.6%) by culture. In the control group, 30 (71.4%) tissue samples were positive by PCR, 31 (73.8%) by microscopic examination, and 28 (66.6%) by culture ($P = 0.34$). Of the 48 blood samples tested over a period of 8 days following *Aspergillus* infection, 14 (58.3%) samples in the control group and 7 (29.1%) in the amphotericin B group were positive ($P < 0$ .05).
Conclusion: The results suggest that this PCR assay is a sensitive tool for detection of *Aspergillus* in infected tissues, independent of an existing therapy. Monitoring of blood cannot be recommended by PCR during amphotericin B application, since negative results did not exclude organ infections.

P544 Combination posaconazole and amphotericin B therapy against pulmonary aspergillosis and systemic candidiasis in mice

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Objectives: Combination posaconazole (POS), a triazole in Phase III clinical trials, has broad-spectrum activity against fungi; amphotericin B (AmB) is the gold standard for treatment of severe invasive fungal infections (SIFI). Combination therapy of SIFI is increasingly important, but there is concern about antagonism between triazoles and AmB.
Methods: (A) Studies with *Aspergillus flavus* (AF) were done in two independent laboratories, using cortisone acetate-compromised mice exposed to AF conidia in inhalation flasks. Treatment was with water (controls), POS alone (10, 2 mg/kg, PO), AmB alone (5, 1 mg/kg, IP) or combinations (POS10 + AmB5, POS2 + AmB1) concomitantly (days 1–7 postinfection, PI) or sequentially (POS days 1–7 PI, AmB days 2–7 PI); 8–12 mice per group. Survival was followed for 8 days (analyzed by log-rank test) and lungs cultured from dead mice daily, and survivors on day 8 (analyzed by Mann–Whitney test). (B) Studies with *Candida albicans* (CA, done only at SPRI) used normal mice infected i.v. with 5×106 or 1×107 CFU per mouse (four strains tested). Treatment with water (control), POS alone, AmB alone, or concomitant POS + AmB began 4 h PI and continued once daily for 4 days, using four-dose levels of each drug in all possible combinations (checkerboard); 10 mice per group. Survival was observed for 10 days (pooled data from two studies per strain analyzed by Wilcoxon's tests).
Results: (A) With AF-infected mice, increased survival and lung burden reductions, relative to controls with POS alone and POS + AmB, were similar, while AmB alone was variable. (B) With CA-infected mice, survival of mice, relative to controls, treated with POS + AmB was either more effective or similar to POS or AmB alone in >99% of 128 comparisons.
Conclusions: POS + AmB combinations were not antagonistic against pulmonary aspergillosis in compromised mice dosed concomitantly or sequentially, or systemic candidiasis in normal mice dosed concomitantly.

P545 *Galleria mellonella*, an in vivo model for the pathogenicity and treatment of *Aspergillus* infections

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Objectives: *Aspergillus* spp. are a frequent cause of infection amongst immunocompromised patients; therefore, the need for more active antifungal drugs is great. In this study, the Greater Wax Moth, *Galleria mellonella*, was investigated as a simple in vivo model for the pathogenicity and treatment of *Aspergillus* infections.
Methods: Wax moth larvae were infected with several strains of *Aspergillus* by inoculation with 10 mL of washed spore suspension (2×103 to 6×106 cfu per larvae, dependent on organism) through the cuticle of the last pro-leg. This inoculum was sufficient to cause death in 90% of the test group between days 3–4. Infected larvae were treated with amphotericin B (AMB), itraconazole (ITZ), or ketoconazole (KTZ) at 0.05 mg/g immediately postinfection, maintained at 30°C, and monitored daily. Control larvae received no treatment and all died between days 3–4.
Results: In this model, pathogenicity is both critically dose and strain dependent, and a hierarchy of pathogenicity was observed as follows: *A. flavus* > *A. niger* > *A. terreus* > *A. fumigatus*. Treatment of *A. fumigatus* (AF210m, AF293) infection was successful with AMB (100 and 80% survival, respectively) and ITZ (100 and 90%), but mortality was high when treated with KTZ (10% survival for both strains). An in vitro and in vivo ITZ resistant strain of *A. fumigatus* was found to be resistant to ITZ (10% survival) in this model also. AMB and KTZ had poor activity against *A. terreus*-infected larvae compared to those treated with ITZ (100% survival).
Conclusion: The data from this study correlate well with both in vitro MIC data and in vivo murine models infected with *Aspergillus*. We therefore conclude that the use of this convenient and inexpensive model could provide a useful tool in the screening of novel compounds for in vivo antifungal activity while avoiding some of the ethical issues and costs associated with other animal models.

P546 Safety and tolerability of caspofungin therapy for elderly patients with invasive candidiasis or invasive aspergillosis

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Background: Elderly patients may be particularly susceptible to drug-related adverse events (DRAEs). Invasive fungal infections have been traditionally treated with amphotericin B (AmB) despite its frequent toxicity and poor tolerability. Caspofungin (CAS) is a new, generally well-tolerated intravenous (i.v.) antifungal drug with activity against *Candida* and *Aspergillus* in vitro and in clinical trials. CAS may provide a useful therapeutic option for elderly patients with IC or IA under some circumstances.
Methods: Patients ≥65 years of age, enrolled in a double-blind, randomized trial of CAS (50 mg/day after a 70 mg loading dose) versus AmB for documented IC, or in an open-label, noncomparative study of CAS for definite or probable IA in patients refractory to and/or intolerant of standard therapy. Patients with IC were to be treated for 14 days after the last positive *Candida* culture; patients responding to i.v. therapy could be switched to oral fluconazole (400 mg/day) after 10 days. Clinical response and status of immunosuppression dictated the duration of CAS therapy for IA patients. Adverse events determined by the investigator to be definitely, probably, or possibly related to CAS were considered to be DRAEs.
Results: There were a total of 58 elderly patients treated with CAS in the two studies (43/114 (38%) IC and 15/90 (17%) IA). Median (range) age was 71 (65–84) years; 53% were men. Nine (60%) of the IA patients were intolerant to other antifungal drugs. Median (range) duration of CAS therapy was 12 (1–23) days for IC and 28 (5–77) days for IA. Fifteen (26%) patients experienced clinical DRAEs; none was serious. Specific clinical DRAEs found in >3% of elderly CAS recipients were phlebitis (5%), nausea (3%), and vomiting (3%). Nine (16%) patients had laboratory DRAEs; none were serious. Specific laboratory DRAEs found in >3% of the elderly CAS recipients were hyperbilirubinemia (4%); elevated direct bilirubin (5%), alkaline phosphatase (7%), and ALT (5%); and decreased serum potassium levels (4%). No one discontinued CAS due to a DRAE. There were no drug-related deaths.
Conclusions: Caspofungin therapy at a dose of 50 mg/day appeared to be generally well tolerated in this subgroup of elderly patients from two studies of invasive fungal infections. The type and frequency of DRAEs in elderly patients treated with CAS were comparable to DRAEs in overall CAS-treated patients. No serious DRAEs or study discontinuations due to DRAEs were noted in 58 elderly patients with IC or IA who received CAS therapy.

P547 Successful treatment of *Trichosporon asahii* infection with caspofungin and liposomal amphotericin B

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Objectives: *Trichosporon asahii* is an emerging fungal pathogen seen particularly in immunologically compromised patients. There are many reported cases of hematogenously disseminated infections with this life-threatening yeast, and no obviously effective antifungal therapy is available. The echinocandins are a promising new class of drugs with broad antifungal activity that demonstrate to be effective in combination with amphotericin B.
Methods: A 54-year-old man was admitted to our hospital on March 2002 for an acute myeloid leukemia (AML). He received two cycles with high-dose chemotherapy with subsequent bone marrow aplasia. After a few days of this treatment, the patient developed fever (Ta_{max}, 39.5°C). He was started in antibiotic therapy as well as in antimicotic therapy. After 1 week, fluconazole was stopped and the patient was started in liposomal amphotericin B (L-AMB) 300 mg daily i.v.; one of the two bottles of blood cultures during the fever was positive for *T. asahii*. After 3 days of L-AMB due to the persistency of positive blood cultures and fever, the patient was started on voriconazole 800 mg i.v. the first day, then 600 mg i.v. During the following days, due to the worsening of the overall conditions, alteration of the renal function, and increase in the bilirubinemia (10.2 mg/dL), the dosage of voriconazole was reduced to 400 mg i.v. daily. During such a therapy, three of the six bottles of blood cultures resulted positive for *T. asahii*. The

antimicogram demonstrated complete sensitivity to AMB, intermediate to fluconazole, and resistance to itraconazole and voriconazole. Therefore, voriconazole was stopped and the patient was started on L-AMB 350 mg daily and caspofungin 70 mg the first day, then 50 mg daily. The condition of the patient continued to deteriorate: bilirubin reached 37.3 mg/dL, creatinin 6.2 mg/dL, azotemia 254 mg/dL, and uric acid 19.7 mg/dL. After 2 days of combination of L-AMB and caspofungin, three bottles of blood cultures were negative. Seven days later, the patient died due to the resurgence of leukemia.
Conclusions: Echinocandins invariably demonstrate high MICs when tested against *Trichosporon* spp. In this case, amphotericin B demonstrated in vitro activity against *T. asahii*, while azoles were resistant. The combination of caspofungin and high-dose L-AMB appear to be an effective treatment solution for the *T. asahii* fungemia.

P548 Successful therapy of cerebral phaeopyphomycosis due to *Ramichloridium mackenziei* with the new triazole posaconazole (SCH56592)

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Background: *R. mackenziei*, a dematiaceous fungus geographically restricted to the Middle East, is a rare cause of cerebral phaeohyphomycosis. There have been no reported cases in which the patient survived despite surgery and antifungal therapy. In an experimental murine model of cerebral *R. mackenziei* infection, posaconazole (POS) was superior to amphotericin B (AMB) and itraconazole (ITZ) in prolonging survival and reducing brain fungal burden, suggesting a possible role of POS for the treatment of this disease.
Objective: To describe a clinical case of phaeohyphomycosis due to *R. mackenziei* that was treated successfully with POS.
Clinical case: A 62-year-old man who had received a kidney transplant 3 months earlier was admitted to the hospital after experiencing left-sided hemiparesis and hemisensory loss. A CT scan of the brain showed a 2×2 cm ring-enhancing lesion in the right parieto-frontal region. Aspirates of the lesion grew a black mould upon culture that was identified as *R. mackenziei*. Despite several weeks of AMB lipid complex 5 mg/kg/day IV and oral ITZ 200 mg twice daily, an MRI showed progression of the original lesion coupled with multiple new satellite lesions. Therapy was switched to liposomal AMB 5 mg/kg/day i.v., oral ITZ 200 mg twice daily, and oral 5-flucytosine (5FC) 25 mg/kg thrice daily. A repeat MRI showed further evidence of disease progression; a craniotomy with evacuation of the large abscess was performed. Minimum inhibitory concentration values of the fungal cultures from the craniotomy specimen were <1 μg/mL for AMB, ITZ, and POS, and 8–16 μg/mL for 5 FC. An MRI 2 weeks later showed enlargement of multiple satellite lesions. At this time, current antifungal drugs were discontinued and POS 800 mg daily (200 mg qid) was initiated. The patient tolerated POS well with no apparent side-effects, and was switched to maintenance therapy with 400 mg bid after initial partial response. Over the next 18 months, several MRIs of the brain showed reduction and subsequent disappearance of the multiple satellite lesions with some enhancement at the original abscess site.
Conclusion: Treatment with POS in this immunocompromised patient resulted in clinically significant symptomatic and radiological improvements after other therapies failed. This case supports the use of POS as salvage therapy for serious dematiaceous fungal infections of the central nervous system and suggests that additional studies are warranted.

P549 Our experience in the treatment of 40 cases with candidal balanoposthitis

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Objectives: The acknowledgement of the real possibilities of the treatment of candidal balanitis and balanoposthitis.
Methods: We analyzed the efficacy of three treatment tactics for 40 cases of age 14–67 years with candidal balanitis or balanoposthitis, observed during March 1995–August 2002. We used three treatment tactics: etiologic treatment only locally; locally etiologic treatment; and oral and surgical treatment when the results were weak, although etiologic therapy. Local treatment: 16 cases were treated with Nizoral cream (Yanssen, ketoconazole 2%, tube of 30 g) applied twice per day for 14 days, 24 cases were treated with Pevaryl cream (Cilag, econazole 1%, tube of 30 g) applied as mentioned above. Oral treatment: 15 cases were treated with Nizoral (Yanssen, ketoconazole in tablets of 200 mg) 2×1 tablet per day for 14 days; with Diflucan (Pfizer, fluconazole in tablets of 150 mg) taking three doses 150 mg within an interval of 10 days, one dose to the next one.
Results: From 24 cases (13 with prepuce), which took only local treatment, 13 cases were cured (four of them with prepuce); also 54.16% of the total were cured and 30.76% of patients with prepuce. For 11 patients (nine cases with prepuce), where local treatment failed, oral treatment was applied, which cured eight patients (four of them with prepuce). So 72.72% of the total and 55.55% of the patients with prepuce were cured. For four patients, where the systematic treatment failed, circumcision was done which solved the problem. While from 16 patients (seven with prepuce), which were treated locally and orally since the beginning, 12 patients were cured (four patients with prepuce), so 75% of the total and 57.14% of those with prepuce were cured. In three cases, where the treatment failed circumcision was successfully applied. Surgery tactic had been altogether applied in seven patients, 17.5% of all cases (two of them with acute balanoposthitis and five with chronic balanoposthitis).
Conclusions: With local antimycotics, 54.16% of all cases or 30.76% of the patients with prepuce were cured; with local antimycotics and oral ones, 72.72 up to 75% of all cases or 55.55 up to 57.14% of patients with prepuce were cured; surgery tactic was necessary on 17.5% of all patients or 9.09% on the acute cases and on 27.77% of the chronic ones.

P550 Treatment of oral candidiasis in HIV/AIDS patients

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Objective: To study breakdown of oral candidiasis, the most common opportunistic fungal infection in HIV-positive persons, with itraconazole capsules, the existing modalities of treatment not yielding satisfactory results.
Methods: Seventy-two persons were selected who are HIV-positive and having oral candidiaisis. Oral candidiasis was diagnosed by fungal culture and KOH smear. Treatment was given with itraconazole capsules 200 mg daily for 15 days. Four patients were dropouts and the rest 56 patients were regularly followed up to 6 months.
Results: All the 56 patients were cured of candidiasis clinically after a mean period of 5 day and mycologically after a mean period of 12 days. Out of the 56 patients who had completed treatment and follow up, eight patients had a reinfection after 4 months. The rest 36 did not have oral candidiasis after a follow up period of 5 months.
Conclusion: As the cure rate (56 out of $72 = 15\%$) of oral candidiasis with itraconazole capsules 200 mg daily for 15 days is convincing, this may be chosen as the treatment for oral candidiasis, which is one of the most common opportunistic infections in HIV/AIDS patients.

P551 Antifungal strategies in febrile neutropenic patients with acute leukemia

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Objectives: The practice of starting empirical antifungal therapy in persistently febrile neutropenic patients is very frequent. Conventional amphotericin B (Con-A) remains the gold standard for suspected or proven fungal infections. Although adverse effects are common, the recent development of liposomal formulations (L-Amb) allows antifungal therapy to be administered with potentially improved efficacy and reduced toxicity. To evaluate the efficacy of L-Amb in persistently febrile high-risk patients or with proven or suspected fungal infections who had not tolerated or not responded to Con-A, febrile episodes in acute leukemic patients were examined.
Methods: Febrile episodes were defined according to EORTC; fungal infections were defined according to Mycoses Study Group. Initially, we used Con–A (1–2 mg/kg/day) and we switched to the L-Amb (1–3 mg/kg/day) if the patient had severe adverse effects; patients who were receiving two or more nephrotoxic drugs concurrently were given L-Amb from the start.
Results: During 1998–2001 period, 149 patients were enrolled and a total of 178 febrile episodes occurred. Of 82 organisms isolated in 73 bacteremias, fungi were responsible for four cases of sepsis (5%). Antifungal therapy was performed in 21 febrile episodes (11%); Con-A was used as initial therapy in 11 patients (pts), L-Amb in 10 patiens. The switching of Con-A to Lip-A was

performed in six patients, the switching of Lip-A to Con-A in one patient (54.5 and 10%, respectively). Evaluable episodes for antifungal therapy were six in Con-A treatment (four FUO, one fungemia due to *C. tropicalis*, one suspected fungal pneumonia) and 15 in Lip-A (six FUO, three fungemia due to *C. famata*, *C. albicans*, *C. Krusei*; five suspected fungal pneumonia, one proven Aspergillosis). Overall, of patients treated with antifungal therapy, successful outcome was achieved in 17 patients (81%): 83% with Con-A, 80% with Lip-A, and death in four patients (19%): 17% with Con-A and 20% with L-Amb. Causes of failure were one FUO in Con-A, two FUO and one *C. Krusei* fungemia in L-Amb. In Con-A treatment, adverse effects were important in hypokalemia in four patients and immediate reaction in two patients; in L-Amb treatment, we documented one acute reaction.
Conclusion: Our results confirm that L-Amb is an effective agent for fungal infections and is far less toxic than conventional form of this drug. However, in some cases, other strategies including high doses of L-Amb or new agents as voriconazole or caspofungin must be considered.

P552 In vitro activity of a new polyene SPK-843 against *Candida* spp., *Cryptococcus neoformans,* and *Aspergillus* spp. clinical isolates

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The most commonly occurring infections among immunocompromised patients are candidiasis, cryptococcosis, and aspergillosis. The treatment of invasive fungal infections has essentially been limited to amphotericin B (AMB) and azoles. Due to dose-limiting nephrotoxicity of AMB and intrinsic or acquired resistance to azoles leading to therapeutic failure, efforts in antifungal pharmacology increased to develop safe and effective drugs. SPK-843, an amide derivative of Partricin A, produced by a mutant strain of *Streptomyces aureofaciens*, has recently been introduced as a promising antifungal agent under investigation, and as less toxic in comparison to AMB. The in vitro fungicidal and fungistatic activity of the new developing water-soluble polyene antifungal SPK-843 was compared with that of AMB against clinically significant *Candida albicans* ($n = 70$), nonalbicans Candida ($n = 39$), *Cryptococcus neoformans* ($n = 49$), and *Aspergillus* spp. ($n = 36$) isolates according to the broth macrodilution reference method for yeasts (M27-A) of NCCLS and the broth macrodilution modification of NCCLS reference method for filamentous fungi (M38-P). Antibiotic medium 3 was used for testing *Candida* and *Aspergillus* spp. isolates and Yeast Nitrogen Base medium for *C. neoformans* strains supplemented with 2% glucose. *C. albicans* ATTCC 90028, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, *C. neoformans* ATCC 90112, and *Paecilomyces variotii* ATCC 22319 were used as quality control strains with known MIC values. MICs were defined as the lowest drug concentration causing 100% inhibition for both test antifungals (MIC-0). The minimal fungicidal concentration (MFC) was determined by plating 100 µL from each MIC drug dilution having no growth or fewer than three colonies onto SDA incubated at 30°C until growth was seen in the growth control subculture. The MFC was the lowest drug concentration having no growth. Five isolates of *C. albicans*, 18 (three of each species) of nonalbicans Candida strains, five of *C. neoformans* and 15 (three of each species) of *Aspergillus* spp. were randomly selected and tested again against each drug to assess the reproducibility of the tests. MIC ranges (micrograms per mililiter) for *C. albicans*, nonalbicans Candida, *C. neoformans*, and *Aspergillus* strains, respectively, were as follows: SPK-843 – 0.007–4, 0.007–8, 0.007–4, and 0.007 to higher than 16 (geometric mean MIC 0.16, 0.28, 0.05, and 0.23 µg/mL, respectively); AMB – 0.015–16, 0.03–16, 0.03–2, and 0.06 to higher than 16 (geometric mean MIC 0.50, 1.01, 0.05, and 0.67 µg/mL, respectively). The MFCs (micrograms per mililiters) for *C. albicans*, nonalbicans Candida, *C. neoformans*, and *Aspergillus* strains were as follows: SPK-843 – 0.03–16, 0.03–16, 0.03–8, and 0.007 to higher than 16 (geometric mean MFC 0.66, 1.01, 0.24, and 0.81 µg/mL, respectively); AMB – 0.125–16, 0.25–16, 0.03–8, and 0.06 to higher than 16 (geometric mean MFC 1.53, 2.22, 0.24, and 1.84 µg/mL, respectively). For all isolates, SPK-843 and AMB were fungicidal in 90.0, 97.1% of *C. albicans*, 89.7, 100% of nonalbicans Candida, 91.8, 97.9% of *C. neoformans*, and 88.6, 94.3% of *Aspergillus* isolates, respectively. The tests were considered valid when the control isolates had the expected values. SPK-843 demonstrated lower in vitro MIC and MFC values than AMB against *Candida* spp. and *C. neoformans* and comparable (species dependent) to those of AMB against *Aspergillus* spp. tested. The data obtained in the present study, are encouraging for further studies including in vitro tests, clinical trials, and animal experiments and accumulation of data will better clarify its therapeutic efficacy in deep mycosis specially due to most prevalent infections caused by *Candida* spp., *C. neoformans*, and *Aspergillus* spp.

P553 Aspects of antifungal action of the novel antimicrobial agent N-chlorotaurine

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Objectives: N-chlorotaurine (NCT, ClHN-CH2-CH2-SO3-), a long-lived oxidant produced by human neutrophils, has broad-spectrum microbicidal activity against bacteria, fungi, and viruses. Bacteria have been demonstrated to lose virulence after short, sublethal incubation times in NCT solution.
Methods and results: Different strains of *Candida* exposed to NCT for sublethal times showed a lag of regrowth in broth media (postantifungal effect). The adherence to epithelial cells was also reduced in *Candida albicans* pretreated with NCT. In the presence of micromolar NCT, the concentration of secreted aspartyl proteases of yeasts decreased, while millimolar NCT killed the fungi. Besides these impacts on virulence factors, there is some evidence that fungal spores treated with NCT for sublethal time undergo enhanced phagocytosis by leukocytes compared to mock-treated ones.
Conclusions: These results provide support that application of NCT in fungal infections may not only act via killing of these pathogens but also via attenuation of their virulence and via synergism with innate immunity.

Emerging infections: problems for the clinician

P554 Invasive *Hemophilus influenzae* disease in Italy due to encapsulated strains other than b

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Objectives: Encapsulated *Hemophilus influenzae* expresses one of the six structurally and antigenically distinct polysaccharide capsules (a–f). Immunization of infants with conjugate vaccine against *H. influenzae* serotype b (Hib) has dramatically decreased the incidence of invasive Hib disease in the developed countries. However, the potential emergence of nonvaccine preventable encapsulated strains other than b, has been suggested. In Italy Hib vaccine was licensed in February 1995 and vaccination is voluntary. The objective of the study was to monitor and characterize the encapsulated strains other than b circulating in Italy.
Methods: In the present study, 198 *H. influenzae* strains, isolated from invasive disease in Italy between June 1997 and September 2002, were tested by a PCR method to determine their capsular genotype. The other than b encapsulated isolates were further analyzed for susceptibility to ampicillin, beta-lactamase production, pulsed-field gel electrophoresis (PFGE) patterns.
Results: By PCR, 128 type b strains, 60 nonencapsulated isolates, 1 b-strain, 5 type e and 4 type f isolates were identified. No type a, c, and d strains were found. All the type e cases occurred in adult or elderly patients, three presenting with bacteremia and two with meningitis, and were detected between January 2000 and December 2001. One of the four type f cases was in a child with meningitis, whereas the remaining three cases, two with bacteremia and one with meningitis, were in adults. The type f cases were detected over the 5-year period. All the isolates were susceptible to ampicillin; none produced beta-lactamase. Two clearly different PFGE patterns were found among the type e isolates. A close genetic relationship was observed among type f strains.
Conclusions: The reporting of nine cases of invasive *H. influenzae* disease caused by serotype other than b strains demonstrated the need for continuous monitoring of invasive *H. influenzae* infection. The large majority of cases due to encapsulated strains other than b occurred in an adult population although one type f case was strikingly reminiscent of the childhood Hib disease. Since, until now, no data on the presence of type e infection have been reported in

Italy, our results suggest the possible emergence of invasive *H. influenzae* type e disease in our country.

P555 A comparative analysis of adult and pediatric infective endocarditis in a developing country

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Objectives: The aim of the study was to compare and contrast the pattern of the disease between the adult and the pediatric age groups.
Methods: All the patients with infective endocarditis admitted between January 1997 and December 2001 to the Aga Khan University Hospital, Karachi, Pakistan were included. SPSS version 11.0 was used for statistical analysis.
Results: A total of 66 patients were admitted between January 1997 and December 2001. Twenty-two patients belonged to the pediatrics age group ($\leq$14 years) and 44 were adults. The mean age for the pediatric and adult patients were 5.8 ± 4.8 and 40 ± 17 years, respectively. Sex ratio was 0.8:1 (M:F) for the pediatric patients while for the adults it was 3.4:1 (M:F). Adult males were more likely to be diseased, and it was a statistically significant difference ($P < 0.05$). Mean duration of symptoms at presentation were 43 and 49 days for the pediatric and adult patients, respectively. Majority of the patients in both the groups presented with fever. A murmur of cardiac origin and splenomegaly were the other common presenting features. Adult patients were more likely to have disease involvement of the native or prosthetic valve while the pediatric patients had more significant involvement of congenital cardiac defects ($P < 0.05$). There were no significant differences in terms of neurologic and renal complications between the two groups. Rheumatic heart disease was documented more commonly as a predisposing condition in adults ($P < 0.05$) and congenital heart diseases were found to be highly significant predisposing conditions in the pediatric patients ($P < 0.001$). Other risk factors were not found to be significantly different between the two groups of patients. There were no differences in the two groups in terms of microbiologic results and the ability of echocardiogram to detect vegetations. However, congenital cardiac defects were more significantly found to be the sites of vegetation in the pediatric age group ($P < 0.05$). Over all in-patient mortality was 27.7% in both the age groups and there were no differences in the clinical outcome.
Conclusion: Our study highlights that pediatric infective endocarditis is a different entity from the adult disease in terms of predisposing conditions and the site of involvement. Rheumatic heart disease was found to be a major risk factor for the adult disease while congenital heart diseases were more common in the pediatric age group.

P556 Hemodialysis patients are not a high-risk group for HTLV I/II infections

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Objectives: Based on the high prevalence of HTLV antibodies, some investigators consider hemodialysis patients as a high-risk group for HTLV infections. Blood transfusions are not in the origin of the infections given the low prevalence in blood donors. On the other hand, we must consider that the enzyme immunoassays generally used for screening anti-HTLV antibodies may give false positive results. If these positive results are not confirmed with more specific tests, such as PCR, the prevalence may be overestimated. Our aim is to study the real prevalence of anti-HTLV in order to consider routine screening for this virus.
Methods: The presence of anti-HTLV I/II antibodies was studied in serum samples using a commercial enzyme immunoassay (Abbott HTLV I/II EIA). The study included 55 hemodialysis patients (mean time in hemodialysis 73.6 months, range 4–278, mean age 60.6 months, range 26–80). Repeatedly reactive samples were further examined by Western blot (WB) and an in-house PCR.
Results: Of the 55 samples tested, only one (0.5%) was repeatedly reactive. When tested by WB, the result was interpreted as indeterminate since only the p24 protein band was present. Interestingly, no nucleic acid was detected by PCR, excluding HTLV I/II infection. Therefore, the actual prevalence of HTLV infections in our study was 0%.
Conclusion: As a consequence of the virtually null prevalence of anti-HTLV antibodies in our country, routine screening is not required in hemodialysis patients. However, we must be aware of the changing epidemiologic situation given the increasing number of immigrants from endemic areas.

P557 Re-emergence of meningococcal disease in Taiwan

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Background: The annual incidence (per 100 000) of meningococcal disease (meningitis and septicemia) in Taiwan was 0.94 in 1953, declined to 0.001 in 1980, and was 0 during the period from1980 to 1987. It re-emerged with a 0.09 (per 100 000) incidence in 1997 and a 0.07 (per 100 000) rate in 2000. From January 2001 to December 2001, a remarkable increase in the number of cases of meningococcal disease (43 cases, 0.19/100 000) was noted.
Methods: The susceptibility of 43 preserved isolates of *Neisseria meningitides* (41 from patients treated in 2001 and one each from patients treated in 1998 and 2000, respectively) to 15 antimicrobial agents was tested by the agar dilution method. Their serogroups were determined by the agglutination method and genotypes were identified by PFGE and random amplified polymorhic DNA patterns. Data on serogroups of 128 isolates from 128 patients with meningococcal disease treated from January 1995 to December 2001 were available.
Results: Among the 128 patients treated from 1995 to 2001, the mean age was 19.4 years and 67 (52.3%) were male. A total of 20 patients (15.6%) died, and the mortality rate was 11.1% in 1995, 30.8% in 1999, and 25.6% in the 2001 outbreak. The majority of the 128 isolates belonged to serogroups B (48.4%) and W135 (35.9%). In 1999, serogroup B isolates accounted for 84.6% of all isolates (11 out of 13 isolates), but in 2001 serogroup B prevalence decreased remarkably (32.6%) while, at the same time, the prevalence of serogroup W135 (41.9%) increased and serogroup Y emerged (18.6%). Three isolates (7.0%) were resistant to penicillin (MICs 0.12 μg/mL), and all were beta-lactamase negative. Among the 43 isolates in 2001, nine domestic clones were identified and four major clones, i.e. clones 1 (serogroup W135, 17 isolates), 3 (serogroup Y, 8 isolates), 4 (serogroup B, 9 isolates), and 5 (serogroup B, 4 isolates) had disseminated in different regions of Taiwan. None of the 43 patients had any relationship (travel or contact history) with the 2000 or 2001 Hajj pilgrimage.
Conclusions: Epidemiologic information and typing results indicate that wide dissemination of a limited number of domestic clones of *N. meningitides*, particularly serogroups W135, B, and Y contributed to the 2001 outbreak in Taiwan. The two clones of serogroup W135 involved in this outbreak were genetically different from the 2000 or 2001 Hajj-related W135 clone.

P558 Leptospirosis in Greece: 1998–2002

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Leptospirosis is a worldwide zoonotic disease caused by pathogenic spirochetes belonging to the genus *Leptospira*. We report the epidemiologic data of leptospirosis cases in Northern Greece, which occurred between the last 5 years (1998–2002). During this time period, a number of 60 hospitalized leptospirosis cases were laboratory diagnosed. The majority of cases were observed in rural areas among males, most of them farmers, or fishers. The age affected was 40–60 years. Cases occurred from June to December, with a peak in August. The disease had usually a severe form with a case fatality rate of 8%. Main signs and symptoms were fever, headache, myalgia, while most of the patients present liver and renal involvement. Jaundice was present in 80% of the cases. Pulmonary involvement was rare. Antileptospiral antibodies (IgM) using ELISA were detected in all of them, while high titers of IgG antibodies were detected in serum samples taken during convalescence. In three cases, early diagnosis of the disease was achieved by PCR; IgM antibodies in these cases were detected 3–5 days later. Leptospiral DNA was detected in a patient's urine sample 65 days after onset of the disease. A number of four serum samples were sent to Institute Pasteur for microscopic agglutination test, and it was found that all of them showed high titers to serovar *L. icterohemorrhagiae*. The main syndrome that is presented with similar symptoms in Greece is hemorrhagic fever with renal syndrome caused by hantaviruses, and differential diagnosis is essential. Treatment included penicillin and supportive management. As weather conditions are changing and global temperature is

increasing, leptospirosis tends to be a re-emerging disease, and precautions have to be taken in order to prevent epidemics.

P559 Re-emergence of acute Q-fever in Israel

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Objectives: Q-fever is a worldwide zoonosis caused by *Coxiella burnetii*, a potential agent of bioterrorism. Although Q-fever is endemic in Israel, the incidence of the disease is unknown.
Methods: We analyzed the epidemiology of all diagnosed cases of acute Q-fever in Israel between 1991 and 2001. These cases were identified through the records of the only two diagnosing laboratories in the country. Demographic data, ethnicity, seasonality, and geographic distribution were retrieved from the laboratory records.
Results: There were 221 patients with median age of 45 years (range: 1–93 years) and a male predominance of 60%. The disease occurred yearly during the study period with an annual number of cases ranging from 4 to 46 (mean: 20 cases), with a peak incidence 58% (129 cases) occurring between May and August. From 1997 to 2001, the annual incidence of Q-fever increased 10-fold (from 0.09 to 0.90 per 100 000 people) among the Jewish population while remained almost unchanged (from 0 to 0.08 per 100 000 people) among the Arab population.
Conclusions: Continuous surveillance and reinclusion of Q-fever on the list of notifiable diseases in Israel are required.

P560 Skin manifestations of 101 cases of murine typhus

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Objectives: *Rickettsia typhi* is the etiologic agent of murine typhus, a zoonotic disease with a worldwide distribution. The main clinical picture of murine typhus includes the classical triad of fever, headache, and a macular or maculopapular rash. The aim of our presentation was to study the skin manifestations in murine typhus.
Methods: One hundred and one patients with compatible clinical status of murine typhus and high serologic titers of antibodies against *R. typhi*, were studied from our team, between January 1993 and December 1998.
Results: Presence of rash (macular or maculopapular) was noted in 81/101 patients (80.1%). Sixty-three out of one hundred and one patients (62.4%) presented with rash on admission, and 18 patients (17.8%) presented skin lesions during hospitalization. The rash was macular in 44/81 patients (54.3%) and maculopapular in 37/81 patients (45.7%). The lesions involved the trunk in 47/81 patients (58.0%), but in 34/81 patients (39%) the extremities were involved. The rash was centrifugal, and the lesions persisted for a period of about 4 days (range: 1–9 days).
Conclusions: The appearance of murine rash represents a clue to the diagnosis of infection from *R. typhi*, but our study showed that in a percentage of 20% there is a delay in its appearance and in a percentage of 20.0% no rash observed. In these cases, only a high index of clinical suspicion can lead the physician to an earlier administration of specific antibiotic treatment.

P561 Blood picture in murine typhus: a study of 101 cases

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Objectives: It is well known that the most frequent hematologic abnormalities in murine typhus (infection from *Rickettsia typhi*) include anemia, an early mild leukopenia and thrombocytopenia. The aim of our presentation was to study the blood picture in murine typhus.
Methods: One hundred and one patients with compatible clinical status of murine typhus and high serologic titers of antibodies against *R. typhi*, were studied from our team, during a period of time between January 1993 and December 1998. Three blood samples were obtained from each patient for the study of their hematologic abnormalities. The first sample was obtained on admission, approximately 9 days after the onset of the disease. The second sample approximately 2 weeks after the first. The third sample, taken from the half of the patients, was obtained 1 month after the second.
Results: On admission (first sample), 31/101 patients (30.6%) presented anemia, 10/101 patients (9.9%) presented leukopenia, and 50/101 patients (49.5%) presented thrombocytopenia. No patient presented leukocytosis. The median value of hemoglobin, white blood cells, and platelets was 12.7 g/dL, 5.9×10^3/mL and 146×10^3/mL, respectively. Two weeks later (second sample) anemia was presented in 57/101 patients (56.4%), 3/101 patients (2.9%) presented leukopenia, 3/83 patients (4%) presented leukocytosis, and 16/101 patients (15.8%) presented thrombocytopenia. The median value of hemoglobin, white blood cells and platelets was 11.4 g/dL, 6.9×10^3/mL, and 218×10^3/mL, respectively. One month later (third sample) 11/46 patients (23.9%) had anemia and 2/46 patients (4.3%) presented thrombocytopenia. No one patient presented leukopenia or leukocytosis. The mean value of hemoglobin, white blood cells, and platelets was 12.5 g/dL, 6.3×10^3/mL, and 224.5×10^3/mL, respectively.
Conclusions: Our study showed that early thrombocytopenia and anemia are frequent in murine typhus, and that white blood cells count is usually normal.

P562 Life-threatening infections due to group B *Streptococcus*: an emerging pathogen in nonpregnant adults

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Background: The incidence of invasive infection due to group B streptococci (GBS) has increased during recent years in nonpregnant adult patients (pts), especially in elderly pts, with chronic immunosuppressive diseases, such alcoholism, diabetes, neoplasia, and HIV infection.
Objective: To present four cases of GBS bacteremia treated in our hospital during the second-half of the year 2002. No case of GBS bacteremia occurred during the last decade in this 750 bed general hospital. Case 1: A 56-year-old-female pt with no history of any underlying disease was admitted because of high fever with chills and four to five daily diarrheal bowel movements. Six out of six blood cultures were positive for GBS. The transesophageal ECHO of the heart revealed aortic valve vegetations. The pt was treated successfully with ceftriaxone (2 g, o.d., i.v. for 6 weeks), but due to severe cardiac failure she undertook valve replacement 1 month after discharge. Colonoscopy revealed a benign polyp of the colon. Case 2: A 68-year-old, diabetic male pt was admitted because of fever and rapidly deteriorating mental status during the previous 24 h. Treatment with ceftriaxone (2 g b.i.d., i.v.) was commenced immediately after spinal tap, suggestive for bacterial meningitis. GBS was isolated from both cerebrospinal fluid and blood cultures. Few hours later the comatose pt was transferred to the ICU, but he died 24 h later. Case 3: A 86-year-old female pt with a history of heart failure and suspect lung cancer was admitted because of severe malaise, fever and cellulitis of the left lower limb. Two out of two blood cultures were positive for GBS. She was treated successfully with cefuroxime (750 mg b.i.d., i.v., for 2 weeks). Case 4: A 62-year-old male pt with a history of alcoholic cirrhosis was admitted because of variceal hemorrhage and aspiration pneumonia. Four out of four blood cultures were positive for GBS. He was treated successfully with piperacillin/tazobactam (4 g/500 mg t.i.d., i.v.). All four GBS strains were susceptible to penicillin.
Conclusion: GBS, a previously uncommon pathogen (except in neonates and pregnant women) represent an emerging pathogen, especially in the elderly. This is in accordance with recent reports from other countries (Clin Infect Dis 2001; 33: 556–61).

P563 Clinical and epidemiologic study of Crimean hemorrhagic fever and hemorrhagic nephroso-nephritis in a region of Bulgaria

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Objective: Crimean hemorrhage fever (CHF) and hemorrhagic nephroso-nephritis (HNN) are serious medical problems due to their grave clinical course, high lethality, and specific epidemiologic distribution.
Materials and methods: Thirteen cases of hemorrhage fever (seven with CHF and six with HNN) were presented. Cases were etiologically proven by

virologic and serologic tests in the National arbo-viral laboratory. Main epidemiologic indicators and basic clinical and laboratory data were studied.
Results and discussion: Four cases (57.17%) of seven with CHF finished lethally. For the country, lethality varies from 11.11 to 14.29%. Most patients tended farm animals in home yard, and they suffered tick bite 10–20 days before clinical symptoms. HNN shows low rate of annual mortality. In the last years, lethal exit is very rare. Males present risk gender group for HNN related to profession – military servants, construction workers in the endemic mountain regions, etc. Average age of 32 years is lower than that of the CHF patient – 54 years. All cases were registered in typical season early spring, summer, and late autumn. All patients come from same endemic for HNN mountainous region. Data shows that focus demonstrates periodical activity. The cases were marked by intensive contamination of habitat with rodents. The major mechanism of infection is alimentary by contaminated drinking water and food. Serious fact is that one of the patients lives in outskirts of Plovdiv. Obviously enduring deratization in endemic regions and populated areas is of great value.
Conclusion: Analysis epidemiologic and clinical data is of great value for early diagnosis. This decreases risk of lethal exit for patients with CHF and HNN. The results demonstrate main directions of efficient epidemiologic control: concrete medical information about reservoirs and mechanisms of transmission of CHF and HNN among population, especially highly risk groups; preventive planned desacarizations and desratizations aimed to regulate population density of vectors and reservoirs in endemic foci. Early diagnosis and aggressive substitution therapy of bleeding as well as hyperimmune gamma-globulin application in CHF and early prevention of cerebral and pulmonary edema in the period of acute kidney failure by artificial clearance methods – hemodyalisis performing in HNN are of most importance for the favorable outcome of the diseases.

P564 The study of prevalence of etiology and outcome of septic shock patients admitted to an obstetric and gynecologic department

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Objectives: The incidence of sepsis, the precursor of septic shock, has continued to increase over past decades. The aim of this study was to evaluate the outcome and etiology of septic shock in Iranian patients.
Methods: During the years 2000–2002, we examined the blood, urine, uterus discharge culture of patients who were known cases of septic shock and the outcome and etiology of the patients were recorded.
Results: Four hundred patients with a mean age of 30 and a range of 25–35 were included. The prevalence of etiology was 299 (74%) septic abortion, 55 (14%) endometritis, 27 (7%) pyelonephritis, and 10 (2.5%) chorioamnionitis, and 172 (43%) died. The results of culture were: *Escherichia coli* (50%), *Klebsiella* (20%), *Enterobacter* sp. (15%), *Streptococcus* sp. (10.5%), and others (4.5%).
Conclusion: According to this study illegal abortion was the most common cause of septic shock, therefore education effective methods of contraception are recommended to women.

P565 Tick-borne encephalitis – outcomes from severe courses

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Objectives: Review of outcomes from the most severe courses of tick-borne encephalitis (TBE) with patients in West Bohemian region in 1992–2001.
Methods: Evaluation of anamnestic data, clinical features, laboratory diagnostic, treatment, complications, sequela, and fatal courses. Analysis based on the review of patient's data admitted on Department of Infectious Diseases, database of Regional Institute of Public Health, summary of year results of virologic laboratory and autopsy protocols. Our set consists of 628 patients (108 below 15 years) with median age of 37.2 years (range: 1–82 years), 63% were male. The severe courses are those with permanent motoric sequela or lethal courses.
Results: We noticed the severe courses with 18 patients (inclusive seven fatal). Encephalomyelitis was a leading clinical feature in all cases. We observed permanent sequela in 11 patient (1.7%) monopareses and parapareses of the upper extremities with eight patients, quadripareses with one female (age of 37 years), hemipareses with one male, cerebellar symptoms with one male. Median age of patients with motoric outcomes is 37.5 years (range: 18–61 years), eight are males. Duration of hospital stay ranges from 6 weeks to 2 years inclusive rehabilitation measures. The median age with lethal cases (seven patients, 1.1%) was 55.5 years (34–69 years), four males. These courses of encephalomyelitis were developed to bulbar symptomatology, respiratory failure, quadrupareses, and impaired consciousness. We noticed the death was more often connected with basic chronic diseases (hypertension, ischemic cardiac disease, diabetes mellitus, obesity, immunodeficiencies (M. Hodgkin)), and with intensive care too (artificial ventilation, treatment with corticosteroids, plasmapheresis). Two patients died due to ventilation pneumonia, two patients of heart failure, one of brain death and autopsy in two patients was not done. The patients died in the period of 20 days till 13 weeks after the beginning of the second stage of disease.
Conclusion: We stated the basic information of severe courses and lethal cases with patients who suffered from TBE. Our patients with chronic internal diseases or persons with extreme load between two stages of disease show higher risk of permanent motoric outcomes and mortality. There is an elementary rule – to be vaccinated against TBE and do not become ill.

P566 Acute disseminated encephalomyelitis associated with *Borrelia burgdorferi*

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Objective: To describe the association between acute disseminated encephalomyelitis (ADEM) and a cerebral infection with *Borrelia burgdorferi* (neuroborreliosis).
Methods: A 45-year-old man developed acute bilateral visual loss, meningism, fever, and paraparesis, followed by a flat tetraparesis, locked-in syndrome, autonomic disregulation, and eventually death. CSF was examined, cerebral MRI and MRA were performed, an extensive work-up for underlying infectious causes was done (using culture, serology, and molecular techniques on blood, CSF, and brain tissue) and finally autopsy was performed.
Results: CSF showed a pleiocytosis (94% granulocytes) and an elevated protein level, but no intrathecal antibody production. MRI/MRA on day 5 demonstrated extensive white matter lesions in frontal and parietal lobes, medulla oblongata, pons, and subcortical areas, compatible with ADEM, while MRI on day 1 only demonstrated two hypodense lesions in the myelum. On Western blot, an IgM response, with a clear *B. burgdorferi* specific 22 kDa band and a weaker 34 kDa band, was present. Infection with known causative agents of ADEM was ruled out. PCR on CSF samples showed *B. burgdorferi* DNA. At histopathologic examination of the brain at autopsy, perivenous demyelinization and perivascular T-lymphocytic infiltration were seen. Finally, PCR for *B. burgdorferi*-DNA of involved brain tissue was positive.
Conclusions: Neuroborreliosis can cause white matter lesions, but the rapid progression, the findings on MRI and the histopathologic results at autopsy in this case best fit ADEM. This disorder is thought to be mediated by autoreactive CNS-specific T cells, that attack shared epitopes of the possible causative microorganism and the myelin. These targets have been demonstrated in patients with neuroborreliosis. To our knowledge this is the first report describing an association between *B. burgdorferi* and ADEM.

P567 Septal panniculitis: a new clinical presentation of acute *Bartonella hensela* infection in a healthy man

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Objective: The disease spectrum of *Bartonella (Rochalimae) henselae* has evolved considerably since the original description of oculoglandular syndrome in 1889. A wide variety of infections may lead to panniculitis, *B. henselae* has been associated with erythema nodosum, however, septal panniculitis in this setting has not been described.
Case report: A 34-year-old white man presented with painful, erythematous nodules involving the proximal upper and lower extremities 8 weeks after he buried two outdoor, domestic cats that had died suddenly. Profound fatigue, myalgia, and joint pain was prominent. He was modestly ill appearing, afebrile, and had generalized lymphadenopathy. An 8 cm arciform lesion

on the right shoulder had bright erythematous borders, and a violaceous center, on histologic examination, severe inflammation of the subcutaneous tissue was accompanied with prominent fat necrosis. White blood counts was 11 700 cells/μL, and a negative workup included, normal serum AST, ALT, ACE levels, ESR, and negative serology for HIV-I, HIV-II, *Borrelia burgdorferi*, *Ehrlichia chaffeensis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Chlamydia trachomatis*, *Coxiella burnetii*, *Brucella* spp., *Yersinia enterocolitica*, and antibodies against native DNA. *B. henselae* IgG titers were markedly elevated (>1024; IgM < 20, by IFA). Detectable serum IgG (256; IgM < 20, by IFA) for *B. quintana* was regarded as intragenus antigen cross-reaction. CT scan of thorax, abdomen, and pelvis, and total body gallium scan were also normal. Response to i.v. azithromycin (AZM; 500 mg) was seen in 96 h and after the third relapse following discontinuation of antimicrobial agent, he received 12 week AZM-therapy and complete response was observed after serum IgG titers (<64 by IFA) had became nondetectable at the end of the 3 month treatment course.

Conclusion: *B. henselae* may be considered in evaluating patients with septal panniculitis.

P568 Tales from the riverbank: *Mycobacterium microti* pulmonary infection mimicking malignancy

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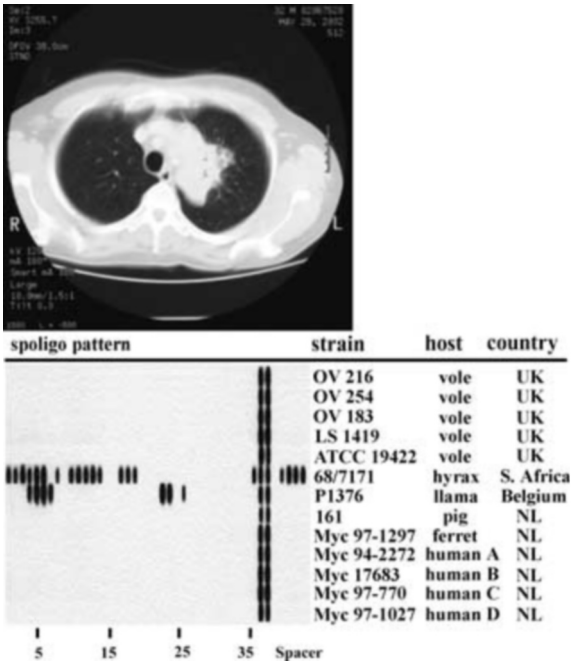
Objectives: We introduce a *Mycobacterium microti* pulmonary infection in an immunosuppressed HIV-positive patient diagnosed with spoligotyping techniques. The treatment of this led to a worsening of his illness, and further CT scanning suggested the diagnosis of pulmonary malignancy. Further investigations were required to exclude this and continue successful anti-mycobacterial treatment.

Method/results: This 33-year-old patient with hemophilia A had been diagnosed with HIV in 1996. He gave a history of 3 months worsening hemoptysis with some minor weight loss but no night sweats. On examination, he appeared moderately unwell. A chest X-ray revealed left upper lobe shadowing and sputum specimens were highly positive for acid-fast bacilli. Blood tests showed CD4 count 46×10^6. Renal and liver functions were normal. He was commenced on broad spectrum anti-mycobacterials with the intention of treating *M. tuberculosis* and *M. avium* complex. He improved and it was decided to commence antiretroviral medication. At a 3 weeks follow-up appointment, he had continued to improve. About 9 weeks after his initial presentation he attended the ward unwell. On examination, he was spiking fevers over 38° and there were new palpable lymph nodes. An urgent CT scan of the chest revealed a 6 cm × 3 cm solid mass reported as 'almost certainly solid neoplasm.' A brochoscopy revealed an area of irregular nodular tissue. A MTB complex PCR performed on the struggling mycobacterial culture was positive. The bronchial biopsy revealed narcotizing granulomatous bronchitis, with no acid-fast bacilli, fungal spores or other organisms. *M. microti* was identified using spoligotyping methods. We think that he had suffered a paradoxical, immune reconstitution reaction. The anti-mycobacterials were reintroduced without problems.



Conclusions: *M. microti* is a slow-growing member of the tuberculosis complex. Its place as a pathogen in humans has been proposed by a handful of case reports. Compared to RFLP, spoligotyping is a PCR-based test that can be quickly and cheaply performed, even on struggling mycobacterial cultures. *M. microti* is an unusual infection associated with small rodents. It has only been introduced as a human pathogen by novel genetic techniques, which begs the question of its true incidence as a cause of pulmonary disease.

P569 Actinomycetoma of the thumb caused by *Gordona terrae*

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In 2001, an 18-year-old patient from Sierra Leone presented himself with a painless lesion on the left thumb (tenar region) that had been slowly progressive for almost 4 years. There were multiple sinuses draining pus and granules. The patient had received no previous treatment, and there were no other complaints. X-ray of the hand showed no bone involvement. Cultures of swabs and granules remained sterile. Surgical debridement was performed. Examination of biopsy material showed sporadic leukocytes with sporadic Gram-positive rods. Culture on sheep-blood agar yielded raised, rough, salmon-colored colonies after 5 days of incubation at CO_2. Gram stain of cultures showed nonbranching Gram-positive rods. ZN/kinyoun stains were negative. Susceptibility testing was performed by disc diffusion on sheep-blood agar. Biochemical determination as *Rhodococcus* species was performed with biochemical tests (Api Coryne, Biomerieux), and *Gordona terrae* (formerly *Rhodococcus terrae*) was identified by means of 16S rRNA gene sequencing. This aerobic, Gram-positive, slightly acid-fast, nonmotile short rod belongs to the family of the Nocardiaceae, genus *Gordona*. It does not form spores or capsules and does usually not produce aerial hyphen. *G. terrae* has been isolated from soil and sputum. Only a few cases of human infection are described. To our knowledge, this is the first description of a mycetoma caused by *G. terrae*. After surgery, the patient received 2 weeks of doxycycline 100 mg once daily with a starting dose of 200 mg. The lesion healed, and no relapse has occurred.

P570 Thigh abscess caused by *Actinomyces neuii* in an adult patient

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Objective: A case of thigh abscess in an adult patient caused by *Actinomyces neui* ssp. anitratus is presented.

Methods: Surgically obtained purulent material from the thigh abscess was inoculated in the appropriate culture media for the detection of both aerobic and anaerobic bacteria. Direct microscopic examination of the clinical material stained with Gram stain was used. Classic methods and API Systems were used for the identification, Kirby Bauer method for the examination of the susceptibility pattern, and *E*-test for the detection of MIC to penicillin G were performed.

Case report: A 62-year-old man with a skin abscess of the left thigh came to the emergencies. In the past, he was treated surgically followed by antibiotic treatment for abscesses located in different sites of his body without any microbiologic examination of the pus. He is a heavy smoker, overweight and 6 months before he was treated surgically for a tibia fracture of the left leg. During the last 2 years, he has suffered from pulmonary disease. He has no fever and he was treated surgically and with antibiotics: cefaclor 500 mg ×3 for 10 days. From the culture of the pus, *A. neuii* ssp. *anitratus* was isolated in pure culture and it was susceptible to penicillin G, ampicillin, amoxycillin/clavulanic acid, cefaclor, cefotaxime, ofloxacine, erythromycin, and resistant to metronidazole. Abundant leukocytes and Gram(+) bacteria were observed at the direct microscopic examination of the sample. Fifteen days later the patient reported automatic rupture of a new abscess of the right thigh. He was

treated surgically and with ampicillin/sulbactam for 10 days. From the culture, *Staphylococcus lugdunensis* was isolated, which was susceptible to ampicillin/sulbactam. We also found a decrease of leukomatines and an increase of a1, a2 and g globulins (inversion), CRP 6.79 mg/L and RF 434 IU/mL.
Conclusions: *Actinomyces neuii* may be the cause of different abscesses and endophthalmitis. In this case, it was thought to be the cause of the infection because it was isolated in pure culture and it was also observed in the direct examination of the clinical specimen.

P571 Increasing incidence of *Stenotrophomonas maltophilia* isolation in a Greek general hospital

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Objective: To study the incidence of *Stenotrophomonas maltophilia* (SM) infection or colonization in patients admitted to Sotiria General Hospital, a tertiary care hospital in Athens, Greece, during 2001–2002.
Results: During the 5-year period 1998–2002, we observed a significant increase in the incidence of SM isolation from clinical specimens from hospitalized patients. From a total of 31 298 isolated microbial strains, 238 strains were characterized as SM. The incidence was increased by sixfold from the beginning to the end of the 5-year period (from 0.2 to 1.8%, respectively). One hundred and seventy-nine (75%) strains were isolated between 1/1/2001 and 30/9/2002. Eighty-six (48%) of the isolated strains were derived from sputum, 48 (27%) from blood, 22 (12%) from bronchial secretions, 12 (6.7%) from pus, 5 (2.8%) from urine, 5 from pleural fluid and 1 from the tip of a central vein catheter. Some of these isolations probably represent colonization rather than true infection. However, the fact that a significant percent of SM strains were isolated from the blood underscores the significance of SM as an emerging nosocomial pathogen. Regarding the antibiotic susceptibility, 97.2% of the strains were susceptible to cotrimoxazole, 90% were susceptible to ciprofloxacin, and 82.7% were susceptible to ticarcillin/clavulanic acid, while 98.4% of the strains were resistant to imipenem/cilastatin. A great variety of antibiotic susceptibility phenotypes was observed, suggesting that there were no epidemic clusters of infection in the hospital. These findings were confirmed in part by applying molecular techniques (PFGE) in a small ($n = 30$) number of strains isolated from blood cultures.
Conclusion: *Stenotrophomonas maltophilia* represents a significant emerging nosocomial pathogen in our hospital.

P572 Nosocomial bacteremia due to *Stenotrophomonas maltophilia*: an analysis of 41 episodes

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Objectives: *Stenotrophomonas maltophilia* is recognized as an important nosocomial pathogen, and in recent studies, it has been increasingly reported as a cause of severe infections. In view of the serious *S. maltophilia* infections that may be encountered in the immunocompromized patients or those patients that are under antibiotic pressure, we evaluated the clinical features and significance of *S. maltophilia* in our patients.
Methods: Forty-one patients with *S. maltophilia* bacteremia were screened out of inpatient population that were admitted to the hospital from various causes between the years 2000 and 2002. Results of this group of patients were compared with those diagnosed as *P. aeruginosa* bacteremia during the same period.
Results: Similar demographic features were noted in both groups. *S. maltophilia* bacteremia was diagnosed on the 17.4 ± 8.8 hospitalization day and *P. aeruginosa* bacteremia was diagnosed on 11.8 ± 5 hospitalization day ($P = 0.001$). The most frequently used antibiotic prior to the bacteremia in the *S. maltophilia* group was determined as carbapenems (61%), and in *P. aeruginosa* group the penicillins (63.4%) were the most commonly used antibiotic. Sensitivity of the isolates of *S. maltophilia* was as follows: cotrimoksazol 95%, TIC/CL 80%, seftazidim 14.8%, ciprofloxacin 14.3%, imipenem 9.4%. Mortality rates due to infection was significantly higher in the *S. maltophilia* bacteremia group ($P = 0.04$).
Conclusions: *S. maltophilia* bacteremia is a severe clinical infection in patients who are exposed to broad-spectrum antibiotics and are admitted in the hospital for prolonged periods. Treatment of *S. maltophilia* is difficult because of its resistance to commonly used broad spectrum antibiotics. Mortality rates due to infection may be decreased by rapid initiation of antibiotics as determined by in vitro active antibiotic studies.

P573 Can we really assert therapy of invasive meningococcal disease (IMD) to prehospital care?

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Objectives: Early prehospital application of antibiotics (PAA) in suspected IMD has been discussed for more than 20 years. Results of many studies were different and most studies proved no statistically significant difference in mortality in groups with and without PAA. One study even proved higher mortality in the group with PAA than without PAA. So, where is the problem?
Methods: We compared results of PAA in patients with IMD in the Czech Republic treated in 1996–2001 (164 patients) – group 1, where mortality in groups with and without PAA was 7.8% vs. 10.4% ($P = 0.55$) and group 2 from West Bohemian region (113 patients), where mortality was 12.5% vs. 20% ($P = 0146$). Survival in both groups was higher in groups with PAA, but not statistically significant difference. During studying of factors, which can play a role in different numbers we found out the difference in the IMD severity in both groups (29% of septic patients in group 1 vs. 51% in group 2). Higher severity of IMD in group 2 corresponds with mortality – 16.2% vs. 9% in group 1. Suspected diagnosis of IMO in prehospital care was established in group 1 in 30% of cases, in group 2 in 55% of cases. PAA was realized mostly in the hospital, rarely by general practitioner or prehospital emergency services. We have experienced that aggressive therapy initiated by prehospital emergency services based on consultation with our center improved survival in IMD. To improve situation in prehospital care, proposal of Standard of prehospital care in IMD was published.
Results: Despite activities to unify principles of complex therapy of IMD in prehospital care, situation in the Czech Republic is not optimal. Neglected primary prehospital care for patients with IMD can facilitate development of severe sepsis with high mortality rate.
Conclusions: Awareness of IMD among all parts of prehospital medical services and conditions for sufficient and complex prehospital care enable to improve survival of patients suffering by this disease. Not only PAA, but complex prehospital care is effective. Permanent system of education controlled by specialized centers can improve this situation.
Acknowledgement: Supported by Grant GACR NI-7109-3.

Mechanisms of resistance 1

P574 Variations in B-lactam susceptibilities between organisms expressing the plasmid-encoded AmpC B-lactamase CMY-2 is attributed to variable gene expression

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Objectives: CMY-2 is a plasmid-encoded (PE) AmpC B-lactamase of *Citrobacter* origin often reported in North America. Current literature suggests discrepancies in MIC data among transconjugants expressing similar AmpCs are greater than what can be explained by experimental variation. The PE blaCMY-2 is not inducible but contains the −35 and −10 promoter elements that drive the expression of the *C. freundii* chromosomal *ampC* gene from which blaCMY-2 originated. The purpose of this study was to identify the promoter regions and RNA expression patterns from two *Klebsiella pneumoniae* (*Kp*) and two *Escherichia coli* (*Ec*) isolates expressing CMY-2 and correlate those findings with MIC patterns and plasmid copy number.
Methods: The strains used in the study were Kp249 and KpV50, and EcL1 and EcB1, all encoding blaCMY-2 in the absence of ESBLs. Susceptibilities to several representative B-lactam antibiotics were determined by agar dilution. A 1.5 kb fragment containing the blaCMY-2 structural gene and 5′-flanking region was amplified by PCR and sequenced. Steady-state RNA expression

levels and transcriptional start sites were determined by primer extension analysis. Gene copy number was determined by comparative PCR analysis.
Results: MICs of the drugs tested against EcL1 were identical or within one dilution to those of KpV50. While susceptibility testing of KpV50 revealed MICs four- to eightfold higher than those determined for Kp249 and EcB1, the RNA expression levels for KpV50, EcL1, and EcB1 were 5.7, 3, and 1.6-fold higher, respectively, compared to RNA expression by Kp249. The gene copy number for KpV50 and EcL1 was four while the gene copy number for Kp249 and EcB1 is two. In addition, the start site of transcription for blaCMY-2 was located 160 bp upstream of the structural gene start codon.
Conclusions: These data indicated that the differences in MICs for these strains reflected the level of RNA expression and correlated with the gene copy number, not variations in the structural enzyme or promoter sequences. In addition, a newly identified promoter was mapped to an insertion element 160 bp upstream of blaCMY-2. Variation in gene expression did not reflect differential expression between strains. These data suggest that variation in MICs observed in the literature may be due to variations in the level of *ampC* gene expression. Therefore, the expression of PE *ampC* genes is an important determinant in the resistance phenotype observed in these strains.

P575 Derepression of inducible plasmid-mediated AmpC beta-lactamase DHA-1 at high mutational frequency after exposure to cephalosporins and aztreonam

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Objectives: Isolates of Enterobacteriaceae possessing plasmid-mediated AmpC beta-lactamases (pAmpCs) can be susceptible in vitro to third generation cephalosporins (3GCs) and aztreonam (ATM). It is unknown if therapy with these agents will be successful or select less susceptible mutants, or how frequently the selection of resistance may occur. Therefore an in vitro study was designed to determine the potential for mutational resistance to emerge in a clinical strain of *Klebsiella pneumoniae* (KP) possessing the inducible pAmpC (ipAmpC) DHA-1 after exposure to various beta-lactam agents.
Methods: The DHA-1 producing strain was susceptible to imipenem (IPM), cefepime (FEP), cefotaxime (CTX), ceftriaxone (CRO), and ATM. It was exposed to (i) increasing concentration gradients of CTX, FEP, ATM, and IPM and (ii) the same agents at concentrations 4×, 8× and 16× above the MIC. Mutants selected were tested by NCCLS agar dilution methodology for changes in susceptibility to the selecting agents and to CRO, piperacillin/tazobactam (P/T), ciprofloxacin (CIP), amikacin, and chloramphenicol (CHL).
Results: ATM and CTX selected mutants derepressed for AmpC production with MICs of ATM, 3GCs and P/T increasing 32- to 512-fold and exceeding resistance breakpoints, while the MICs of IPM and FEP remained unchanged or only moderately increased (two- to eightfold), and remained in the susceptible range. The derepressed mutants selected by CTX and ATM occured at high mutational frequencies of approximately 10^{-5}. FEP also selected a derepressed mutant but only with the gradient plate method (i.e. frequency $<10^{-9}$). Unlike the 3GCs and ATM, IPM did not select any derepressed mutants but instead selected a mutant that was more resistant to CHL.
Conclusions: These data indicate that this ipAmpC producing strain of KP can readily develop broad beta-lactam resistance on exposure to 3GCs and ATM, and that FEP can also select derepressed mutants but less readily. The findings shed more light on what may occur during therapy of infections caused by pathogens that harbor ipAmpCs.

P576 Analysis of the *blaVIM* gene context from Italy reveals novel resistant cassettes: report from the SENTRY Antimicrobial Surveillance Program

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Objective: To analyze isolates with metallo-beta-lactamase (MBL) genes, both *blaIMP* and *blaVIM*, reported in Italy since 1999. Very little is known of the variation with respect to their genetic context. Therefore, as part of the SENTRY Antimicrobial Surveillance Program, we genetically analyzed 22 *Pseudomonas aeruginosa* isolates possessing MBLs from three different Italian cities.
Methods: For amplification of IMP, VIM, and SPM genes and the adjacent genes intI1, aacA4, aph, aadA1, and qacED1, PCR was performed using AB-gene Expand Hi-fidelity master mix PFU/proof reading TAQ polymerase and dNTPs. Primers were designed within the middle of each gene for a given cassette to examine the genetic context of the MBL gene. Sequencing of PCR amplicons was undertaken on both strands by the dideoxy-chain termination method. Sequence analysis was performed using the Lasergene DNASTAR software package. Alignments and phylogenetic analysis was obtained using Clustal W and PAM 250 matrix.
Results: Positive PCR products from screening known MBL genes were found in 16/16 strains (*blaVIM*) from Genoa and 4/4 strains from Catonia (*blaVIM*), but no PCR products were found from the two isolates from Rome. Sequencing of the *blaVIM* PCR products from all sites showed it to be *blaVIM-1*. The genetic context of *blaVIM* was assessed by PCR and showed different gene arrangements to that previously reported. Of the 16 isolates from Genoa, one isolate (75–3634) gave a PCR pattern indicating that the 3′ end of the cassette had been deleted and that this casette consists of intI1, blaVIM-1 and aacA4 only. One isolate from Catonia (85–2394) gave a PCR pattern indicating that the cassette no longer carries the *aph* gene.
Conclusions: *P. aeruginosa* strains 75–3634 and 85–2394 carry novel blaVIM-1 cassettes. These data clearly indicates that while most mobile genetic constructs are stable, some are undergoing genetic rearrangements resulting in either gene replacement or gene deletion within epidemic or endemic regions for MBLs.

P577 Cloning of a hybrid metallo-beta-lactamase gene conferring a novel IMP variant blaIMP-13 in *Pseudomonas aeruginosa* in Italy: report from the SENTRY Antimicrobial Surveillance Program

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Objective: To analyze the determinant of carbapenem (imipenem and meropenem) resistance in a clinical strain of *Pseudomonas aeruginosa* isolated in Rome as part of the SENTRY Antimicrobial Surveillance Program in 2001.
Methods: A gene bank of *P. aeruginosa* strain 86-1457I genomic DNA was constructed in the cloning vector pK18 using standard molecular biology techniques. The amplified gene bank was used to transform *Escherichia coli* DH5-a and plated on media containing both ceftazidime and the serine lactamase inhibitor BRL42715. All clones isolated harbored the same plasmid (pMATPsRM). The insert of pMATPsRM (1.8 kb) was sequenced on both strands by the dideoxy-chain termination method with a Perkin Elmer Biosystems 377 DNA sequencer, and sequence analysis was performed using DNAstar software.
Results: The clones when expressed in *E. coli* conferred low-level ceftazidime resistance (4 mg/L). Sequence analysis revealed the presence of a metallo-beta-lactamase gene that belonged to the blaIMP family, designated blaIMP-13. The amino acid sequence of IMP-13 diverges from the amino acid of IMP-1 by 14.5% after the first 90 residues of the N-terminus. Functional changes of particular interest and not present in any other IMP metallo-beta-lactamase were G138E, Y181H, and N236K. Near the N-terminus of the truncated IMP-13 (three residues away from the zinc binding motif HFHSD) was situated the LacZ forming a IMP-LacZ hybrid protein. All clones containing blaIMP-13 contained the truncated gene and the fused LacZ/IMP-13 protein in exactly the same location. Truncated IMP-13 demonstrated a weak metallo-beta-lactamase activity.
Conclusions: *P. aeruginosa* strain 81-11963 A contains a novel metallo-beta-lactamase gene, blaIMP-13. All clones containing blaIMP-13 contained a truncated form of the protein that could still produce a protein with detectable metallo-beta-lactamase activity.

P578 Acquisition or evolution of the SHV beta-lactamase gene on the *Klebsiella pneumoniae* chromosome?

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Objectives: It is widely accepted that the SHV beta-lactamase gene evolved on the chromosome of *Klebsiella pneumoniae*. The evidence for this includes the finding that over 95% of *K. pneumoniae* strains carry SHV, but that very few

other members of the genus carry the gene, except on plasmids. In order to test this theory, we examined the location and type of beta-lactamase gene in a plasmid-deficient strain of *K. pneumoniae.*
Methods: *K. pneumoniae* strain MGH78578 was isolated at Massachusetts General Hospital from the sputum of a 66-year-old male patient confined to the ICU in 1994. Genome sequence for this strain was examined for the presence of any sequences related to known beta-lactamase genes using http://genome.wustl.edu/gsc/Blast/client.pl.
Results: Genome sequence analysis revealed that MGH78578 carries an SHV-2 beta-lactamase gene. The gene is clearly chromosomal because overall context analysis indicates the presence of typical 'housekeeping' genes in the vicinity of SHV-2. The local context of the gene, however, reveals the presence of an IS26 element, the associated transposase gene, and multiple copies of the IS26 Right and Left repeats. Searches for this region of the MGH78578 chromosome in general nucleotide databases revealed fragments of the SHV-2 chromosomal region in a number of SHV-2 containing plasmids found in organisms such as *Salmonella enterica* sv. Typhimurium, *Shigella flexneri* and *Pseudomonas aeruginosa*. When present in plasmids, the SHV-2 region from *K. pneumoniae* MGH78578 is often jumbled, and the IS26 element is not always present.
Conclusions: SHV-2 is chromosomal in *K. pneumoniae* MGH78578. This does not mean, however, that the gene evolved in this organism. It could be that the gene was carried onto the chromosome from a plasmid as part of IS26. Alternatively, the presence of IS26 next to SHV-2 on the chromosome of *K. pneumoniae* MGH78578 could explain the prolific mobilization of this gene onto plasmids. Whether the insertion of SHV-2 onto the *K. pneumoniae* chromosome, or indeed the insertion of IS26, thus allowing SHV-2 mobilization, is an ancient event remains to be seen.

P579 Characterization of an unusual extended-spectrum beta-lactamase from *Proteus mirabilis*

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Objectives: The aim of this study was to identify the extended-spectrum beta-lactamase produced by a *Proteus mirabilis* strain from an adopted Indian child with urinary tract infection.
Methods: Two strains of *P. mirabilis* were isolated from two urine samples from an Indian child adopted by a Spanish family; one was isolated in June 1999 and the other in December 1999. The susceptibility profile of the two *P. mirabilis* was determined using automated microdilution MicroScan method, and production of ESBL activity was identified using double-disk synergy test with two cephalosporins: ceftazidime and cefotaxime. Beta-lactamases were extracted by ultrasonication, supernatants containing crude enzyme extracts were analyzed by isoelectric focussing on ampholine polyacrylamide gels (pH 3.5–9.5) using a Multiphor II electrophoresis System (Pharmacia Biotech), and betalactamases were visualized with an overlay of nitrocefin.
Results: The two isolates were highly resistant to ampicillin (MIC > 16 mg/L), ticarcillin (MIC > 64 mg/L), cefazoline (MIC > 16 mg/L), cefuroxime (MIC > 16 mg/L), cefotaxime (MIC > 32 mg/L), ceftazidime (MIC > 16 mg/L), and cefepime (MIC > 16 mg/L), but susceptible to amoxicillin-clavulanic acid (MIC < 4/2 mg/L) and cefoxitin (MIC < 8 mg/L). They were also resistant to gentamicin (MIC > 8 mg/L), tobramycin (MIC > 8 mg/L), amikacin (MIC > 16 mg/L), trimethoprim-sulphamethoxazole (MIC > 2/38 mg/L), and fosfomycin. Synergy was observed between clavulanic acid and cefotaxime and ceftazidime. Isoelectric focussing analysis revealed the presence of two beta-lactamases with pI of 7.6 and approximately 5.5.
Conclusions: The presence of ESBL-producing *P. mirabilis* is increasing, and getting an important epidemiologic and geographical variability. Detection of strains with mechanisms of resistance infrequent in European countries should be considered in immigrant patients or in adopted children.

P580 Resistance to third-generation cephalosporins and putative ESBL production in a urinary isolate of *Salmonella enterica* serovar Virchow

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Objectives: Extended spectrum beta-lactamases (ESBL) were first detected in *Klebsiella pneumoniae*, but in the recent years they have been reported with growing incidence in other Enterobacteriaceae taxa. In the *Salmonella* genus, ESBLs have been detected mainly in serovars Typhimurium and Enteritidis. Here we report ESBL production in a urinary isolate of *Salmonella enterica* serovar Virchow.
Methods: A 13-year-old girl had her spinal cord damaged during spinal surgery for scoliosis, turned out to be paraplegic and was intermittently catheterized. Her urine samples were routinely sent to our laboratory, where a *S. enterica* serovar Virchow was isolated in November 2002. Antimicrobial susceptibility testing was performed by disk diffusion and interpreted according to the NCCLS. The presence of an ESBL was investigated by means of a double-disk synergy test agar, of the combined disk method and of *E*-test.
Results: The identification was confirmed at the species and serovar level at Hifzisihha Institute. The isolate proved resistant to cephalosporins, including the third-generation compounds. The presence of an ESBL was also suggested by the synergies detected between clavulanic acid and cefotaxime, ceftazidime, or aztreonam in the disk diffusion assay, with a typical deformation of the inhibition zone, as well in the other tests performed. The isolate was resistant to trimethoprim-sulfamethoxazole, but susceptible to quinolones and aminoglycosides. The patient had no symptom of urinary tract infection. She had no previous gastrointestinal disease; her stool cultures were negative, and ultrasonography of her gallbladder did not show any evidence that she was a chronic carrier of the microorganism.
Conclusions: To our best knowledge this is the second published report of *S. enterica* serovar Virchow producing ESBL. The only previous report was about one strain isolated from stools of four Spanish patients with gastroenteritis, and in that case the ESBL was identified as CTX-M-9. The fact that our strain was more resistant to cefotaxime than to ceftazidime suggests the possibility of a cefotaximase type enzyme, as well.

P581 Extended-spectrum beta-lactamase producers among Enterobacteriaceae of patients in Bulgarian hospitals

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Methods: Forty-one strains identified as ESBL producers (*Klebsiella pneumoniae*, 26; *K. oxytoca*, 1; *Escherichia coli*, 8; *Citrobacter freundii*, 2; *Enterobacter cloacae*, 4) were collected from four medical centers in Sofia and Pleven during 2000–2002. MICs were determined by an agar dilution technique according to NCCLS guidelines. Further analysis included conjugative plasmid transfer and isoelectric focusing.
Results: MIC of ceftazidime ranged from 8 to >512 mg/L and of that cefotaxime from 2 to 512 mg/L. For all strains, MIC of ceftazidime was higher or equal to the MIC of cefotaxime. In all strains, sulbactam in combination with ceftazidime and cefotaxime showed an inhibitory effect. Ceftazidime resistance was transferrable in 35 strains. There are two clusters of strains. One cluster (33 strains of *Klebsiella* and *Enterobacter*) demonstrated three different beta-lactamases with isoelectric points of 5.4–5.6 (TEM-type), 7.0–7.6, and 8.2 (SHV-type). Transconjugants from this strains on IEF had bands at 5.4–5.6 and 8.2. Another cluster (eight strains of *Klebsiella*, *Citrobacter*, *E. coli*) demonstrated beta-lactamases focussing between 6.3 and 6.5, which suggest TEM type. The pI data were from transconjugants and wild-type strains. *Enterobacter*, *Citrobacter* showed additional bands suggested chromosomal beta lactamases.
Conclusion: During the investigation period, ESBL of the SHV- and TEM-type were dominating among Enterobacteriaceae in Bulgarian hospitals.

P582 The characteristics of aminoglycosides resistance in nosocomial Gram-negative strains in Russia

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Objectives: To determine phenotypes and mechanisms of resistance to aminoglycosides in nosocomial Gram-negative strains in ICUs in Russia.
Methods: A total of 648 nosocomial Gram-negative aminoglycoside-resistant strains isolated in ICUs of 14 Russian hospitals were examined. The samples were sputum, wound samples, abdominal drains, urine, and blood. The mechanisms of aminoglycoside resistance were determined by aminoglycoside resistant pattern (AGRP) method. The following aminoglycosides were used: kanamycin, neomycin, gentamicin, tobramycin, netilmicin, isepamicin,

amikacin, 5-epi-sisomicin, 2′-*N*-ethyl-netilmicin, 6′-*N*-ethyl-netilmicin, fortimicin, apramycin, lividomycin, and butirosin.
Results: The predominant phenotypes of aminoglycosides resistance were: gentamicin-tobramycin-netilmicin (44.9%), gentamicin-tobramycin (22.2%), and gentamicin-tobramycin-netilmicin-amikacin (13.8%). The gentamicin-tobramycin-amikacin-isepamicin phenotype was determined only in 4.2% of examined strains and gentamicin-tobramycin-netilmicin-amikacin-isepamicin phenotype was found in 4% of strains. The most frequent mechanism of resistance was the production of aminoglycoside modifying enzymes. The prevalent enzymes were: AAC(3)-V, 46.9%; ANT(2′), 27.9%; AAC(6′)-I, 11.4%; APH(3′)-VI, 11,9%. The rare enzymes were: AAC(3)-I, 2.5%; AAC(3)-IV, 1.2%; AAC(2′)-I, 1.1%; AAC(3)-III, 0.6%. The majority of tested strains produced 2–6 enzymes simultaneously (83.5%), including the APH(3′)-I enzyme, causing resistance to kanamycin and neomycin.
Conclusions: The leading mechanism of aminoglycoside resistance in Russian Gram-negative nosocomial strains was the production of aminoglycoside modifying enzymes. According to the obtained data, determination of aminoglycoside resistance mechanisms in given hospital is essential for the rational choice of aminoglycoside for empirical therapy of nosocomial infections.

P583 Diversity of IMP-type metallo-beta-lactamases in carbapenem-resistant clinical isolates of *Pseudomonas* spp. from Italy

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Objectives: The production of an acquired metallo-beta-lactamase (MBL), mainly in *Pseudomonas* and *Acinetobacter* species, confers a broad-spectrum resistance to nearly all beta-lactam antibiotics, including third- and fourth-generation cephalosporins, and carbapenems, that is not reversible by mechanism-based inactivators. The lack of clinically useful inhibitors and the mobility of MBL-encoding genes promoting their spread among important opportunistic pathogens also account for their notable relevance in the clinical setting. The IMP-type MBLs represent a heterogeneous family of closely related variants, encoded by mobile gene cassettes inserted into integrons, that have been reported from the Far East, Europe, North and South America. Here we report a molecular characterization of clinical isolates of *Pseudomonas* spp. producing IMP-type MBLs collected in Italy, in the period 1999–2002.
Methods: Carbapenem-resistant clinical isolates were screened for MBL production by molecular methods (multiplex PCR, colony blot) and by phenotypic tests (EPI microdilution test). The nature of the determinants and their genetic background were determined by DNA sequencing of PCR-amplified cassette arrays of blaIMP-carrying integrons.
Results: Four IMP producers were detected among a total of 24 MBL-producing isolates of *Pseudomonas* spp., two *Pseudomonas aeruginosa* producing the IMP-2 enzyme and one *Pseudomonas putida* producing the IMP-12 enzyme from the University Hospital of Varese (northern Italy), and one *P. aeruginosa* producing a new IMP-variant, named IMP-13, from the Hospital of Atri (central Italy). The different blaIMP determinants were encoded by gene cassettes of different phylogenies that were carried by integrons of heterogeneous structure. Apart from blaIMP-12, that was carried by a nonconjugative medium-sized plasmid, the other determinants appeared to be located on the chromosome. The new IMP-13 enzyme shares 85% identity with IMP-1, and is closer to IMP-2 and IMP-8 (92–93% identity).
Conclusions: blaIMP genes are rarely detected among MBL-producing clinical isolates from Italy. A remarkable heterogeneity of enzymes structure and genetic background was found. The sequence of a new IMP-type determinant, named IMP-13, quite divergent from any other known IMP-type determinant, is reported.

P584 Molecular characterization of integrons from *Klebsiella pneumoniae*

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Introduction: Integrons are genetic elements that contain the determinants of a recombination system that is able to capture gene cassettes, most frequently antibiotic resistance genes, such as aminoglycoside-modifying enzymes and beta-lactamases. Integron-driven gene capture probably extends beyond the dissemination of antibiotic resistance genes to the more general process of evolution of bacterial genomes.
Objective: Molecular characterization of integrons from *Klebsiella pneumoniae* isolates.
Methods: Five multiresistant strains of *K. pneumoniae* collected between 1980 and 1999 at a University Hospital in Lisboa were selected for this study. Total DNA preparations were used in PCR experiments with specific primers for each class of integrons and/or gene cassettes. For each strain, the amplicons obtained from three independent reactions were sequenced on both strands.
Results: All isolates revealed the presence of one or two integrons, which contained at least one antibiotic resistance gene cassette, belonging to classes 1 or 3. The two isolates from 1980, resistant to streptomycin, presented a class 1 integron with an aadA1 gene cassette, similar to that previously found in Tn21 from *Shigella flexneri*. In the strain isolated in 1991 was found a class 1 integron with one gene cassette similar to dfrV, which conferred resistance to trimethoprim. The isolate collected in 1995, presented two integrons, one identical to that found in the strain from 1991, and a class 3 integron, with a GES-1-like gene cassette. The isolate collected in 1999 presented only a class 3 integron with two gene cassettes, whose genes encoded a GES-1 extended-spectrum beta-lactamase and an AAC(6′)-I enzyme conferring resistance to kanamycin.
Conclusions: Two different classes of integrons were found in the *K. pneumoniae* strains. The same strain showed different classes of integrons, class 1 and class 3. The same class 1 integron was found in strains isolated in 1991 and 1995, suggesting a degree of stability of these genetic elements over a 4 year period. This report provides further evidence of the wide distribution of integrons and their potential to contribute to antibiotic resistance.

P585 Characterization of antimicrobial resistance and integrons in *Salmonella* from Portugal

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Objectives: The purpose of this study was to determine the antimicrobial resistance patterns of *Salmonella* of different origins, and to detect and characterize class I integrons, in order to identify the resistance genes located therein.
Methods: This study included a total of 100 isolates of *Salmonella* collected in Portugal during 2002. Isolates were from human ($n = 27$) and nonhuman ($n = 73$) (foodborne, waterborne and environmental) sources, and belonged to several serogroups. The MICs for 10 antimicrobial agents were determined by the agar dilution method, and the results were analyzed according to the NCCLS standards. The presence of class I integrons were tested by PCR, using the primers 5′CS−3′CS, for all the sulfamethoxazole resistant isolates ($n = 24$) and for 29 isolates with other susceptibility profiles. The isolates with one or more amplicons were also tested for the presence of *sul1* gene, using 5′CS-sul1B primers. The 5′CS primer was used in combination with several primers for antibiotic resistance genes (*ant-(3″)*, *pse-1*, and *dhfr-1*) to determine the content of the variable regions of the integrons.
Results: Seventy-five percent of the isolates were resistant to one or more of the antimicrobial agents tested. Resistance to nalidixic acid (36%), tetracycline (35%), streptomycin (33%), sulfamethoxazole (24%), ampicillin (23%), trimethoprim (17%) and chloramphenicol (11%) was observed. The most frequent multiresistant phenotype, observed in nine isolates (serogroups B and C1), was to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT), found alone or simultaneously with trimethoprim. Class I integrons were present in 19 multiresistant isolates (serogroups B and C1), which ranged in size between about 1000–2000 bp. In four isolates (serogroup B), two different integrons (1000 and 1200 bp) were simultaneously identified. The *sul1* gene was localized in all the integron types obtained in the present study. The *ant-(3″)* gene, alone or in combination with other resistance genes, was found in variable regions of 18 integrons. Furthermore, gene cassettes encoding the *pse-1* and *dhfr-1* gene were found in several integrons.
Conclusions: Our study of Portuguese isolates suggests a high incidence of multidrug resistance in *Salmonella* of different origins and a possible role for integrons in the acquisition of resistance genes.

P586 Detection and characterization of class 1 and 2 integrons in *Shigella* strains isolated in Senegal

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Objectives: This study aimed to evaluate the contribution of integrons in multi resistant strains of *Shigella*.
Methods: Thirty *Shigella* strains belonging to serotypes flexneri ($n = 14$), dysenteriae A1($n = 13$), and sonnei ($n = 3$), and six *Shigella* strains which were not agglutinated with classic antisera were isolated from 1997 to 2000 from diarrheal adult patients in an university teaching hospital and an urban hospital, in Dakar. Strains were resistant to ampicillin, ticarcillin, tetracycline, trimethoprim-sulfamethoxazole, and chloramphenicol. Integrons belonging to classes 1, 2, and 3 were screened for by PCR using primers specific for *intI1*, *intI2*, and *intI3* genes. Gene cassettes were identified by sequencing.
Results: Integrons were detected in 30 strains (83%): 12 strains contained a class 1 integron, four strains contained a class 2 integron, and 14 strains contained two integrons, one of class 1, and one of class 2. All strains belonging to an unknown serotype contained both class 1 and 2 integrons. No class 3 integron was detected. Eleven class 1 integrons do not contain the open reading frames usually found in the 3′ segment (qacED1, sul1 and ORF5). For class 1 integrons, the size of the inserted fragment varied from 0.7 to 1.8 kb. Seven strains had an integron that contained a single cassette dfrA5 or dfrA15, both conferring resistance to trimethoprim. The remaining class 1 integrons contained two cassettes: oxa1-aadA1a, dfrA15-aadA1a, dfrA1-aadA2, oxa30-aadA1a. For class 2 integrons, strains carried the same three cassettes as those found in Tn7 (dfrA1, sat and aadA1), but in most cases, the aadA1 cassette was missing. Eleven RAPD profiles were identified: three among *Shigella dysenteriae* A1, two among *S. flexneri*, two among *S. sonnei*, and four among *Shigella* spp. The integron carriage was unrelated to the RAPD profile.
Conclusions: Various integron structures were detected in 30 out of 36 clinical isolates of *Shigella* indicating that these elements are largely spread among multiresistant strains of *Shigella*. *dfr* gene cassettes were highly prevalent; this is due in part to the intensive use of trimethoprim in combination with sulfonamide to treat diarrheal illnesses in Senegal.

P587 Mechanism of resistance to nalidixic acid in *Aeromonas* spp. clinical isolates

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Objective: To investigate the mechanisms of resistance to nalidixic acid in strains of *Aeromonas* spp. isolated from different clinical sources.
Methods: Fourteen clinical isolates of *Aeromonas* spp. (one Amedia, eight Averonii biotype sobria, three Acaviae, and two Ahydrophila) recovered from different clinical sources were identified to species level by 16S rDNA-RFLP, while the biotype of the *A. veronii* isolates was determined on the basis of the arginine dihydrolase positive reaction and negative response to bilis-aesculin hydrolysis and ornitine decarboxylase production. The MICs of nalidixic acid and ciprofloxacin were determined by the *E*-test method. Finally, the presence of mutations in the *gyrA* and *parC* genes was analyzed by PCR and sequencing.
Results: Ten out of 11 nalidixic acid resistant isolates presented a mutation in position 83 of the *gyrA* gene resulting in the amino acid substitution Ser to Ile in five isolates, and Ser to Arg in the other five. The remaining isolate presented a mutation in position 80 of *parC* gene generating the amino acid change Ser to Ile. No differences were observed in the levels of MIC of nalidixic acid and ciprofloxacin between the isolate presenting a single mutation in *parC* gene and those presenting a single mutation in the *gyrA* gene. None of those 11 isolates presented resistance to ciprofloxacin, but all had a decreased susceptibility to this antimicrobial agent with MICs ranging from 0.047 to 0.25 mg/L. No mutation was found in either the *gyrA* or *parC* genes was found among the three nalidixic acid susceptible isolates.
Conclusions: Mutations in the *gyrA* gene are the most relevant mechanism of nalidixic acid-resistance among *Aeromonas* spp. In addition, the presence of a single mutation in parC gene might confer similar level of resistance to nalidixic acid to that produced by the mutation in the *gyrA* gene.

P588 Zinc eluted from siliconized latex urinary catheters decreases OprD expression causing carbapenem resistance in *Pseudomonas aeruginosa*

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Objective: The activity of carbapenems against *Pseudomonas aeruginosa* (*Pae*) decreases in the presence of siliconized latex urinary catheters (SLUCs). This effect is not due to drug inactivation or increased beta-lactamase activity, but it is associated with the loss of an OprD-like protein and the expression of a new outer membrane protein (OMP) of about 50 kDa. This study was undertaken to investigate which of the SLUC componets are responsible of the decreased carbapenem activity against *Pae* grown in the presence of SLUCs.
Methods: Segments of SLUCs were analyzed for elements by a particle-induced-X-ray-emission technique (PIXE). Elements were also investigated in cation-adjusted Mueller–Hinton broth (MH) and in eluate from SLUCs (four 1 cm segments in length/mL of MH, 37°C, 24 h) by inductively coupled plasma atomic emission spectrometry (ICP-AES). MICs of imipenem (IPM) (Merck, Sharp and Dhome, Madrid, Spain) against Pae PAO1 and its OprD-deficient mutant were determined by a microdilution assay according to NCCLS guidelines. OMP profiles were determined by SDS–PAGE, and further analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry after in-gel trypsin digestion.
Results: PIXE analysis revealed that zinc was the most abundant element found in SLUCs, followed by silicon, phosphorus, sulfur, and chlorine. Finally, traces of copper, titanium, chromium and manganese were also detected. Zn content in MH and in eluate was 0.29 and 36.8 mg/L, respectively, as determined by ICP-AES. Zn concentrations in MH lower than 5.7 mg/L did not affect IPM activity against *Pae* PAO1, while Zn concentrations between 5.7 and 18.9 mg/L determined a fourfold increase in the MIC. IPM activity in eluate and in MH supplemented with Zn to a concentration equivalent to that found in eluate (36.8 mg/L) was the same (MIC: 8 mg/L). On the other hand, MICs of IPM against the OprD-deficient mutant were the same (8 mg/L) in all the media tested. Both *Pae* PAO1 and its OprD-deficient mutant showed the same OMP profile when grown in either eluate or Zn-supplemented MH. Analysis of tryptic fragments from the OMP lost in eluate and in Zn-supplemented MH fitted OprD (formerly OprD2), and the new OMP expressed in those media was identified as OprD3.
Conclusion: Zn eluted from siliconized latex urinary catheters inhibits OprD2 expression, the porin responsible for carbapenem entry into *Pae*.

P589 Dissemination and characterization of integrons type 1 among epidemiologically unrelated clinical isolates of *Acinetobacter baumannii* from Spanish hospitals

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GEIH-AB 2001 project

Objectives: To study the presence of integrons type 1 in 71 strains of *Acinetobacter baumannii*, resistant to beta-lactam antibiotics and aminoglycosides, isolated from 28 hospitals of Spain in November, 2000.
Methods: To achieve this aim, susceptibility testing to these antibiotics were carried out by a microdilution method. The epidemiologic relationship was determined in previous studies by REP-PCR and PFGE. PCR amplification of Class I integrons and DNA sequencing were done using primers and conditions previously described.
Results: Of a total of 71 epidemiologically unrelated strains of *A. baumannii*, 21 (29.58%) presented this type of integrons. Fifteen out of the 21 strains with integrons (71.42%) showed the presence of a 750 bp product which contained a single *aadB* allele encoding an AAD(2″)-Ia aminoglycoside adenyltransferase. Three out of the 21 strains (14.28%) yielded an amplification product of approximately 2200 bp. Direct sequencing of the amplicon revealed the presence of three gene cassettes, the first containing an *aacA4* allele encoding an AAC(6′)-Ib aminoglycoside acetyltransferase, the second containing an ORF coding for a yet undetermined product named orfO and found also in other integrons, and the third containing the *blaoxa-20* gene. Two out of the 21 strains (9.52%) gave an amplification product of approximately 800 bp.

Direct sequencing of this amplicon revealed the presence of a single gene cassette that contained an *aacA4* allele encoding an aminoglycoside 6′-*N*-acetyltransferase AAC(6′)-Ib which was identical to that found in the above-mentioned integron. To finish, only one isolate (4.76%) showed an amplicon of approximately 2500 bp containing four gene cassettes. The first cassette contained an aacC1 determinant encoding an AAC(3)-Ia aminoglycoside acetyltransferase, followed by two open reading frames (ORFs) coding for unknown products that are carried by two cassettes. The fourth cassette contained an aadA1a determinant encoding an AAD(3″)-Ia aminoglycoside adenyltransferase.
Conclusions: Our results confirm the high prevalence of integrons found in *A. baumannii* by other authors and also show the potential risk of integron dissemination among genotypically distinguishable strains of this microorganism.

P590 Plasmid profiles from one strains of *Aeromonas caviae* isolated from fish

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Objectives: The aim of this study was to determine plasmid-mediated tetracycline resistance in one strains of *Aeromonas caviae* isolated from fish and comparative between two methods to detect the plasmid present in this strain and to calculate precisely the molecular weight of the plasmid.
Material and methods: Resistant *Aeromonas* strain were incubated in mixed culture with mutant *Escherichia coli* 'DH5 alpha' recipient strains susceptible to tetracycline. Then, the strain was examined for the loss of resistance phenotypes by curing with ethidium bromide (EB) at 5, 10, 20, 40, 80, 180, and 320 μg/mL concentrations and SDS 10 g/L. Plasmid DNA was isolated by the modified method of Kado and Liu and Holmes and Quigley and separated by horizontal electrophoresis at 150 V, 110 mA for 5 h.
Results: The results of tetracycline-resistant plasmid profiles show size of plasmid was 10 kb (approximately 6×10^6 molecular weight), curable and transferable plasmid was detected. Comparison between Holmes and Quigley methods with Kado and Liu modification methods used to detect plasmids in this study yielded evidence that the best resolution of plasmid bands and repeatable were achieved with the methods of Kado and Liu.
Conclusions: Results analysis confirmed that loss of resistance phenotype was accompanied by loss of plasmid DNA. This study highlights the potential pathogenicity and growing incidence of resistance within clinical and environmental isolates of *Aeromonas*. The increasing use of antibiotics in the human population, animal and pisciculture have provided antimicrobial resistance in soil and aquatic microorganisms. Moreover, the aquatic and gut bacteria come in frequent intimate contact with other organisms bearing transferable R-plasmids.

P591 Induction of multiple antibiotic resistance in *Escherichia coli* strains by diazepam, phenobarbital, and valproic acid

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Objectives: Salicylate (SL), sodium benzoate (SB), and other compounds may induce marRAB transcription in *Escherichia coli* leading to an increased MarA protein expression (a 15 kDa cytoplasmic protein), OmpF loss, and multiple antibiotic resistance (Mar). Diazepam (DZ), phenobarbital (PB) and valproic acid (VP) are used in the treatment of epilepsy. Urinary infections are frequent in children with epilepsy. DZ, PB, and VP are mainly excreted by the renal route unaltered or conjugated. Their role as marRAB inducers has not been previously studied. The objective of the present study was to compare the effect of DZ, PB and VP with SL and SB on the induction of Mar in *E. coli* strains.
Methods: Higher, lower and equal concentrations to the mean therapeutical doses per day (MTD) of DZ, PB, and VP were assayed to determine their inducing effect of Mar in the Ag100 and KL16 *E. coli* strains. The Ag112 (marR mutant) and JF703 (deficient in OmpF) strains were used as controls. Cefoxitin (FOX), nalidixic acid (NA), norfloxacin (NOR), tetracycline (TE), and chloramphenicol (CL) MICs were determined with and without 5 mM SL, 20 mM SB, DZ, PB, and VP. The effect of 5 mM SL, 20 mM SB, DZ, PB, and VP in the outer membrane proteins (OMPs) was analyzed by electrophoresis in SDS–PAGE. The expression of MarA was studied in crude cell extracts (CCE) and analyzed by SDS–PAGE.
Results: The presence of 5 mM VP (in the range of MTD), 5 mM PB, and 0.25 mM DZ (four- to tenfold maximum MTD) or higher concentrations of these just like SL and SB increased the FOX, NA, NOR, TE, and CL MICs from two- to eightfold and led to OmpF loss in the Ag100 and KL16 strains. Electrophoresis of CCE from the Ag100 strain obtained after incubation with the above-mentioned concentrations of DZ, PB, VP, SL, and SB and CCE from the Ag112 strain showed a 15-kDa protein which was absent in CCE from the Ag100 strain grown without DZ, PB, VP, SL, or SB. Mar phenotype induction by DZ, PB, VP, SL, or SB was always associated with concentrations of these compounds which were close (two- to fourfold lower) to their respective MICs in the assayed strains.
Conclusions: DZ, PB, and VP induced a Mar phenotype in *E. coli* strains similar to that which was derived from marRAB activation by SL or SB. Only DZ, PB, VP, SL, or SB concentrations, which were close to their respective MICs in the assayed strains, induced Mar phenotype.

P592 Elimination of R-plasmids under influence of the pulsed electrical discharge

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Objectives: Study of influence of pulsed electric discharges (PED) on stability of inheritance R-plasmids in antibiotic-resistant strains of *Staphylococcus aureus*.
Methods: For research, 20 methicillin-resistant strains of *S. aureus* (MRSA) were selected, allocated from the surgical patients with pyoinflammatory diseases of the bottom respiratory ways that described plasmid resistance to tetracycline, kanamycin and ryfampycin. Processing PED of 10 mL bacterial suspension (10^9 CFU/mL) spent during 5, 10, and 15 s with the minimal frequency of following pulses, then made sowing on meat–peptonic agar. In clinical isolates and cultures which have undergone the influence of PED, minimal inhibitory concentrations (MIC) of antibiotics were determined by methods of agar dilution, and presence of plasmids was determined by a method of electrophoresis in agarous gel.
Results: PED is characterized by presence of a complex of the factors, major of which are: a shock hydraulic wave, ultraviolet irradiation, ultrasonic cavitation, local increase of temperature, generation of the active forms of oxygen, and also minimal concentration of metals, emulsified from a surface of electrodes as a result of electroerosion. At processing PED, the bacterial cell is exposed to powerful stressful influence that at long-term influence (more than 10 s) is shown by destruction of a cell, and at short-term influence (5 s) promotes loss of stability of inheritance R-plasmides by bacterial cell, that was confirmed by authentic decrease in MIC (to kanamycin and ryfampycin <2 mg/mL, to tetracycline <4 mg/mL). The comparison of plasmids structures of strains before processing PED has revealed loss of R-plasmids by cells subjected to influence.
Conclusions: At short-term influence PED on MRSA cells, the infringement of stability of inheritance R-plasmids is observed.

P593 Sequence analysis of *dhfr, dhps* and *pfcrt* genes in *Plasmodium falciparum* isolates from school children in Iringa Rural District, Tanzania

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Introduction: The resistance of *Plasmodium falciparum* to sulfadoxine-pyrimetamine (SP) is linked to mutations occurring in the genes coding for dihydrofolate reductase (DHFR) and for dihydropteroate synthetase (DHPS). Their effect on the SP resistance phenotype has been well established. Differently, the mechanism of *P. falciparum* chloroquine resistance has not yet been clearly elucitated, and several candidate genes have been proposed so far (*pfmdr1*, *cg2*). Recently, a new transporter gene, the *pfcrt* has been identified, and the mutations detected in his nucleotide sequence were always detected in resistant *P. falciparum* isolates in a number of studies.
Objectives: Within the framework of the Italy/Tanzania malaria control project, a survey was carried out in six villages of Iringa district. Resistance level to antifolates in *P. falciparum* by sequence analysis of dihydropteroate

synthetase and dihydrofolate reductase among school children was determined. Chloroquine resistance was also analyzed in view of its possible extended use by population in spite of the antimalarial drug policy adopted.
Methods: A molecular screening on *dhfr*, *dhps*, *pfcrt* genes was carried out on *P. falciparum* isolates. Plasmodial DNA was extracted from blood smears by a QIAGEN kit, and from IsoCode Stix according to the manufacturer's instruction. Molecular diagnosis on all samples was done. Samples confirmed positives for *P. falciparum* were submitted to PCR with primers specific for each target gene. The amplicons from *dhfr*, *dhps* and *pfcrt* genes of 718, 748, and 211 bp, respectively, that contain the most important point mutations were sequenced at the MWG Biotech and the results analyzed by dedicated computer program.
Results: The results relevant to a total of 47 *P. falciparum* isolates from the study area were reported: 108-DHFR mutant alleles account for 74% (32/43) of the isolates screened. On the contrary, 436 and 437 DHPS mutant alleles account for only 14.8% (4/27) and 22.2% (6/27), respectively. no. 76-PfCRT mutant alleles have been detected in the 10 *P. falciparum* isolates so far screened for this codon.

Viral and parasitic infections in children

P594 Comparison of serum amyloid A protein and C-reactive protein concentrations in hospitalized children with viral infections

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Objectives: To investigate the clinical significance of serum amyloid A (SAA) in viral diseases and relation between SAA and C-reactive protein (CRP) in hospitalized children.
Methods: We examined 95 children with viral infections including HSV-1, EBV, CMV, ECHOVIRUSpool, COXSACKIE B1-6, RSV, 20 with *Mycoplasma pneumoniae* pneumonia and 25 as healthy controls. The children aged 6 months−14 years old (mean 6.2 years). Viral infections were diagnosed according clinical findings and serologic evaluations. SAA and CRP concentrations were determined by using Latex agglutination nephelometric immunoassay. Student's *t*-test and regression analysis were used for statistical analysis.
Results: In 95 out of 115 patients (92.5%) the SAA concentrations became markedly raised in the acute phase of viral infection: EBV (81%), CMV (84.3%), HSN-1 (79.3%), ECHIVIRUSpool (85.4%), COSXACKIE B1-5 (84.3%), RSV (75.8%). In convalescence phase of the above infections, the SAA concentrations were normal (<5 μg/mL), also in Mycoplasma infections and healthy controls ($P = 0005$). In 46% of the patients with acute viral infections had raised SAA concentrations (>5 μg/mL) with normal CRP levels (<5 μg/mL). In patients with *M. pneumoniae* the CRP levels were found increased in 58.4%, while SAA in 56.2%.
Conclusions: (i) The increase of SAA concentrations may provide a useful marker of viral infection in cases where the differential diagnosis may be difficult clinically. (ii) In acute viral infection the combination of SAA and CRP could be useful to confirm the diagnosis of the infection.

P595 Echovirus serotype 13 infection in children in Lithuania

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Background: The morbidity of enterovirus infections has increased in Lithuania during summer and fall in 2001.
Aim: To establish the frequency of enterovirus isolation in hospitalized children with viral meningitis or nonspecific rashes during epidemic period.
Patients and methods: Thirty children, aged 1.5 months to 14 years, referred to hospital during August–September in 2001 with initial diagnosis of viral meningitis or nonspecific rashes. Information included personal and epidemiologic data, signs and symptoms, laboratory results, initial diagnosis. Viral cultures from feces, throat and cerebrospinal fluid (CSF) were collected.
Results: Eight (26.9%) of 30 children had positive results of viral culture. Echovirus serotype 13 was isolated from feces (eight children) and throat (one child), but was not detected from samples of CSF. All eight children had fever for 2.3 days. Two patients had nonspecific rashes, 6 viral meningitis. All children recovered.
Conclusions: In Lithuania enterovirus infection was associated with echovirus serotype 13 during epidemic period in 2001.

P596 Significance of the IgG titration against mumps virus for diagnosis of the infection in vaccinated children

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Objectives: To evaluate the application of IgM detection and IgG titration as diagnostic markers of mumps, according to the vaccination status.
Methods: Serum samples of 64 cases of clinical parotitis (mean age 8.1 years; standard deviation 7.0) and of 310 asymptomatic controls (mean age 6.0 years; standard deviation 3.3) were studied. The vaccination status (number of administered vaccine doses and strain, Rubini 'R' or Jeryl Lynn 'JL') was available in all cases and controls. Determinations of IgM in cases and IgG in cases and controls were carried out by indirect ELISA (Enzygnost IgG and IgM, Dade Behring).
Results: In 27 of the 64 cases mumps specific gM was detected (global sensitivity 42.2%). The sensitivity of IgM in relation to the vaccination status was: 100% in non vaccines (12/12), 42.1% in vaccines with one dose of R (8/19), 25% in vaccines with one dose of JL (5/20), 18.2% in vaccines with one dose of R plus 1 of JL (2/11) and 0% in vaccines with two doses of JL (0/2). The sensitivity of different IgG cut-off titers, according to the vaccination status (in all cases with specificity higher tan 95%) were as follows: zero doses (IgG titer 4250) sensitivity 41.7%; one dose (R) (IgG titer 2250) sensitivity 94.7%; one dose (JL) (IgG titer 6000) sensitivity 75%; two doses (1R + 1JL) (IgG titer 10 250) sensitivity 36.4%; two doses (R) non applicable; two doses (JL) IgG titer 13 000 sensitivity 0%.
Conclusions: The detection of mumps specific IgM is the method of choice for mumps diagnosis in unvaccinated patients. However, in vaccinated children, the diagnosis based on the detection of IgM is not enough sensitive. In these instances, the titration of IgG could be an useful complementary tool for the diagnosis of this infection, especially in individuals vaccinated with the Rubini strain. According to our results the definition of confirmed case of mumps must be reviewed.

P597 Tick-borne relapsing fever in Ardabil, the main endemic area in Iran: an epidemiologic survey

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Objectives and background: The main area of Iran affected by relapsing fever (RF) is Ardabil province, in which *Borrelia persica* is the most common cause. First presentation of *B. persica* and *B. baltazardi* to world was from Ardabil in Iran. The aim of this study was to determine the epidemiologic characteristics of the disease, and frequency of infection among ticks in this region.
Methods: This clinical epidemiologic and entomologic study was performed on a total of 391 patients diagnosed tick-borne RF from 1998 to 2001. The presence of *Borrelia* with any species as well as the clinical characteristics was observed. *Borrelia* was searched the thick blood smears of 1421 ticks collected from the 130 indoor human and animal shelters and 14 outdoor places studied. The ticks were crushed and the suspension obtained was injected into the peritoneum of mice and pigs to determine the infection rate among collected ticks. Data including the tick spices were collected through a questionnaire, and analyzed using Chi-square and ANOVA tests.

Results: Forty-nine percent of cases were females and 51% were males. 84.3% of the cases were children and those who did not work outside their homes. The mean age of the patients was 12.111.2 years. The most frequent clinical manifestations were fever, chills, and headache. Other findings included nausea, vomiting, sweating abdominal pain, arthralgia, cough, photophobia, eosinophilia, hematuria, jaundice, petechiae and scleral congestion. Chills were 10% more frequent in females than males. Laboratory tests performed on 60 patients showed leukocytosis, high erythrocyte sedimentation rate (ESR), and anemia. Regardless of sex, hemoglobin level was less than 11 mg/dL in 34% of cases. Of the 1421 ticks collected, 45.9, 40.3 and 13.8% were of the *Ornithodorus lahorensisi*, *Ornithodorus tholozani* and *Argas persicus* species, respectively. The ticks collected from three villages were found to be infected with *Borrelia*.
Conclusion: The clinical manifestations were similar to those reported in other studies. Petechiae occurred less frequently in our study compared to louse-borne R.F. The high frequency of anemia, which was not stated in most other reference articles, requires further investigation.

P598 Rubella epidemiology in Arkhangelsk region, Russian Federation, in the prevaccination era

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Objectives: The main task of this study was to investigate situation with rubella incidence in the North of the European part of the Russian Federation before the vaccination program establishment and discover epidemiologic peculiarities of rubella in Arkhangelsk region.
Methods: We used data on rubella reported to the State Sanitary-Epidemiological Surveillance Center in Arkhangelsk region. Descriptive and statistical methods for data proceeding were used in the investigation.
Results: The maximum rubella incidence per 100 000 population was discovered in 1985 (659), 1990 (471) and 1999 (1211). School children and kids attending kindergartens predominated. Maximum incidence was revealed at the age from 3- to 5-year-old with gradual reduction of the figures, and the second lower incidence peak was discovered at the age of 14–15 years old. Most cases occurred in the winter and spring time, with the peak in April (14.5% from the total during the year). For the observation period there were no cases of congenital rubella documented, while there are some cases in other regions of Russia.
Conclusions: According to the received data, rubella incidence in Arkhangelsk region has a waveform character. The degree of increase during the following peak correlate with the duration of the interpeak period. This is caused by the cumulative growth of nonimmune individuals between epidemic years. Age distribution of the incidence reflects the picture, which is characteristic for the behavior of this infection in other regions. Seasonal rubella distribution is similar to the seasonality of other airborne infections. Relative extension of high incidence period during a year is probably connected to the more severe weather conditions in Arkhangelsk region. A high number of rubella cases were observed in organized collectives such as schools and kindergartens. This is probably caused by the increased possibilities of airborne transmission in these crowded areas. This analysis of the epidemiology of rubella in Arkhangelsk region was taken into the account when planning population vaccination.

P599 Pediatric visceral leishmaniasis in Albania

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Introduction: Visceral leishmaniasis is an endemic disease in northern and north-eastern part of Albania, but sporadic cases are seen even in the other regions of the country. Pediatric age is the most affected by this infection considering immunologic characteristic of this age group. The university hospital center is the only reference hospital for all over the country concerning this disease.
Objectives: To see the trend of pediatric visceral leishmaniasis during the quinquennial 1995–2000. To show the epidemiologic characteristics of this important infection.
Patients and methods: This is a retrospective study based on the analyzing of all medical records of children with visceral leishmaniasis hospitalized at our clinic during the 5-year period June 1995–June 2000. Epidemiologic data included were: age, sex, place, seasonality, outcome and case mortality rate.
Results: During the study period, 457 children with visceral leishmaniasis, aged 6 month to 14-year-old were admitted to our clinic. The distribution of the patients for each year was as follows; first year (June 1995–96) enrolled 70 (15.3%) patients, second year—77 (17%) patients, third year—103 (22.5%), fourth year—121 (26.5%) and during fifth year—86 (18.7%) children were registered. An increase of cases during last 3 years was observed. The disease was most spread in summer. Rural origin (65%) and the northern part of the country dominated among the other regions. The age group 1–4 years was most infected (74%), followed by the infants (14%). Children above 4 years presented 12% of the hospitalized cases. There was a slight domination of male patients (56%). Case fatality rate was 2.4%, five (1.1%) cases presented resistance to the treatment with Meglutamine antimoniate (Glucantime) and four (0.9%) cases progressed toward chronic hepatitis. The others had a satisfied complete recovery, although during the clinic course of the disease different complications such as bronchopneumonia, enteritis, measles were observed.
Conclusions: Visceral leishmaniasis is an important infection for some Albanian regions. The number of pediatric cases has an increasing trend. Glucantime still remains an efficacious treatment for this age group.

P600 Visceral leishmaniasis in Greek children, 1996–2002

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Visceral leishmaniasis constitutes the most frequent form of leishmaniasis, particularly in Mediterranean area. In Greece the precise prevalence rate among children remains unknown. In this study 18 children <14 years (14 males−4 females: 3.5/1) were included, who were diagnosed having visceral leismmaniasis in the Pediatric Clinic of University Hospital of Ioannina, during a 7-year period (1996–2002). The patients mean age was 4.6 years, from which 11 (61%) were <5 years. Ten patients (55%) came from Albania and eight from Greece, area Epirus (three from capital city and five from the wider region) and the symptoms appeared in 61% of them, during spring and summer. They presented with classical clinical manifestations: 17/18 with fever and splenomegaly, 16/18 with hepatomegaly, anorexia and weakness, 8/18 with enlarged lymph nodes, vomiting, diarrhea and cough, 5/18 with bruises and petechiae and one child with jaundice. In addition all of them presented with hypergammaglobulinemia, 94% with anemia, 72% with leucopenia, 66.6% with thrombocytopenia, while the 11% had pathologic chest X-ray. In the whole group of children a control of antibodies against leismania was done, with the methods of indirect immunofluorescence (bio-Merieux) and indirect (passive) hemagglutination (Dade-Behring) and with a positivity rate: 1/40–1/1280 and 1/8–1/1024, respectively. Eighteen out of 18 patients were found positive with the IFA and 14/18 with the IHA. In addition in the 15/18 patients (83%) bone marrow aspiration was done and in the 11/15 (75%) leishmanias were found. All patients were treated with meglumine antimonite intramuscularly for 21 days, without any clinical relapse.
Conclusions: The children in which visceral leishmaniasis was diagnosed, primarily by the presence of antibodies against leishmania, were usually under five years, boys, who came mainly from agricultural regions. They presented with classical symptoms, the majority during spring and summer and responded very well to suitable therapy, with no clinical relapse.

P601 Presentation on admission of WHO-defined severe malaria in children aged 6–59 months in two district hospitals

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More than one century after the discovery of the Plasmodium parasite malaria remains a major cause of morbidity and mortality in the world with 2.3 billion people are exposed (41% of the world's population), there are 300–500 million clinical cases and 1.5–2.7 deaths annually. Children less than 5 years old carry the largest part of this burden with 3000 deaths daily. Knowledge of the various forms of severe malaria at peripheral hospitals is important in order to better direct available resources for case-management. Hence we set out to describe the clinical and paraclinical presentation at two peripheral hospitals in the Central province, Cameroon; this according to the current criteria of the

World Health Organization (WHO). From January 1 to August 31 2000, at the Djoungolo and the Mfou district hospitals 148 children aged from 6 to 59 months who presented with at least one feature of severe malaria were recruited by consecutive sampling. The incidence of severe malaria was 21.1%. The male/female ratio was 1.06. Fever (96.6%), anorexia (62.2%) and convulsions (54.7%) were the commonest symptoms, while prostration (88.5%), confusion or drowsiness (70.3%), inability to feed (68.9%), pallor (56.8%) and splenomegaly (48.0%) were the commonest signs in these children who arrived at the hospital 4 days on average after the onset of illness. Most (79.7%) children were said to have received antimalarial drugs before admission and chloroquine was the most presumed drug of choice (73.4%). The paraclinical presentation was marked by a high median parasitemia at 18000/mm^3. This high parasitemia was not influenced by drugs taken before admission. The commonest clinical forms of severe malaria were: generalized convulsions (54.7%), prostration (43.2%) and severe anemia (14.9%). We recommend reinforcement of education of parents/caretakers on the management of malaria at home and the equipment of peripheral referral hospitals with transfusion facilities in order to reduce the number of referrals to the central level of transfusion-requiring cases.

P602 Evolution and outcome on case management of severe malaria following the current WHO guidelines in children in two district hospitals in Cameroon

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Malaria is a major endemic parasitic disease which remains the main cause of morbidity and mortality in sub-Saharan Africa. Children less than 5 years old carry the largest part of this burden with 3000 deaths daily. Case management of severe malaria is a main problem in Cameroon. The regime proposed by the WHO needed to be tested in African countries to prove its effectiveness and efficacy before adoption. This is thus part of a multicenter study. Our main aim was to describe the evolution and outcome of severe malaria on management following the current WHO guidelines. From January 1 to August 31 2000, 148 children aged from 6 to 59 months with at least one feature of severe malaria were recruited by consecutive sampling, at the Djoungolo and the Mfou district hospitals. Treatment according to WHO guidelines was implemented and there was rigorous in-patient monitoring and the outpatient follow-up. The incidence of severe malaria was 21.1%. The male/female ratio was 1.06. The commonest clinical forms of severe malaria were: generalized convulsions (54.7%), prostration (43.2%) and severe anemia (14.9%). The case management of severe malaria was effective for, the mean fever clearance time was 27.9 ± 21.4 h, the mean coma recovery time was 36.0 ± 17.0 h, the parasitemia reduced by 96.5% 48 h after onset of treatment and the hematocrit increased from $26.4\% \pm 6.7$ initially to $33.7\% \pm 3.7$ on day 28. Most children (95.9%) were completely cured, 2.0% died and there were no neurologic deficit over 1 month follow-up. We recommend the generalization of the protocol throughout the national territory in Cameroon and further training of health personnel to facilitate the utilization of the protocol.

P603 Assessment of the relation between trace elements and antioxidant status in children with protein energy malnutrition

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Background: Protein energy malnutrition represents one of the commonest nutritional deficiencies in developing countries. Marasmus is a severe form of protein energy malnutrition and is associated with oxidative hemolytic anemia. The oxidative hemolysis could be associated with reduced level of antioxidant enzymes (e.g. superoxide dismutase [SOD] and glutathione peroxidase [GPX]) and some trace elements, which serve either cofactors for these enzymes or integral parts of them, e.g. zinc, copper, and selenium.
Objective: The current study was performed to evaluate the level of those antioxidant enzymes and some trace elements in different degree of marasmus and their correlation.
Patients and methods: This study was carried on 30 patients suffering from marasmus (14 patients with 1st degree marasmus and 16 patients with 2nd degree marasmus). They were selected from infants and children admitted to mansoura university children's hospital. Twelve healthy children of matched age and sex were included in the study as a control group. All patients and controls were subjected to: determination of RBCs level of SOD, whole blood level of GPX enzymes, and serum level of zinc, copper, selenium, lead, manganese, nickel, and chromium.
Results: Significant decrease of RBCs SOD, and whole blood GPX in the marasmic groups compared with the control group ($902.45 + 80.87$, $25.87 + 3.39$ for the 1st degree marasmus group, $662.14 + 135.57$, $18.01 + 2.95$ for the 2nd degree marasmus group and $1666.47 + 205.58$, $56.04 + 11.58$ for the control group, $P = 0005$. Also, there is significant decrease of the serum level of copper, zinc, and selenium in both groups of marasmus compared with control group. Serum level of nickel, manganese, and chromium show significant increase in both groups of the marasmus compared with control group.
Conclusions:

1. Decreased activities of antioxidant enzymes in marasmic children, which make them liable to toxic effect of reactive oxygen species.
2. Low serum levels of some micronutrients as selenium, copper, and zinc in marasmic children.
3. There are an existing correlation between serum levels of micronutrients and blood levels of antioxidant enzymes which reflect the importance of these trace elements in the process of tissues protection against oxidative stress.
4. High level of lead, chromium, and nickel in both control and diseased children may reflect environmental pollution.

Microbial pathogenesis

P604 *Aspergillus fumigatus* elicits a complement response in a liver cell line (HepG2 cells)

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Objective: Fungal infections are an increasing problem and can be fatal in immunocompromised patients. The complement system with complement factor 3 (C3) plays a major role in a fungal invasion. A change in C3 RNA expression can therefore be used to monitor an impending immune response. We have used HepG2 cells as a model system in order to see how liver cells respond to filamentous fungi and a modulation with soluble beta-1,3-glucan, a component of the cell wall of *Aspergillus fumigatus* (AF).
Methods: HepG2 cells were stimulated with various concentrations of LPS and conidia of AF with and without beta-1,3-glucan. RNA expression was analyzed by RT-PCR, C3 protein was measured by ELISA.
Results: Both LPS and AF elicited an increase in C3 RNA. The LPS-dependent increase occurred faster and was more pronounced than the C3 RNA increase seen after AF stimulation. Soluble beta-1,3-glucan had a lower effect on C3 expression during the same time period. In combination with AF (preincubation with beta-1,3-glucan (1 h) and stimulation with AF (8 h), C3 RNA expression was clearly inhibited compared to the reverse stimulation combination. LPS-dependent C3 secretion was nearly constant during the stimulation period and seemed unaffected by its high RNA expression response. In contrast, AF-dependent C3 secretion showed a bell-shaped curve but with an inverse shape; starting and ending with a concentration of secreted protein slightly above control, but falling to 50% of control at 18 h of stimulation. Protein secretion after combinations of AF with soluble beta-1,3-glucan showed again an inverse correlation, high C3 expression coinciding with low protein secretion.
Conclusion: Both LPS and AF are able to elicit a substantial increase in C3 RNA expression while the increase in C3 protein secretion was less

pronounced. Soluble beta-1,3-glucan was able to dampen an AF- dependent C3 RNA increase. Protein secretion was different; while LPS stimulation showed a near constant secretion pattern, independent of C3 expression, did protein secretion after AF stimulation as well as after combination with beta-1,3-glucan follow its C3 expression curve, but in an inverse manner.

P605 Effect of IL-1 and IL-10 on uropathogenic *E. coli* adherence to human endothelial cells

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Objectives: The polyfunctional effect of IL-1 on endothelial cells has already been well documented. Present study aimed at analyzing of IL-1 and IL-10 effects on adherence of uropathogenic *E. coli* to human endothelial cells.
Methods: The studies were performed using *E. coli* strains which were isolated from urine of adult patients. The strains were identified as belonging to *E. coli* species using Api ID 32 GN strip and the ATB system (bioMerieux). All the strains were assayed by agglutination using the latex reagent for detection of *P. fimbriae* (*P. fimbriae* particle agglutination test). Adherence of bacterial cells to human umbilical vein endothelial cells (HUVEC) was studied after labeling of bacteria with BrdU. Bacterial adherence was detected by immunoenzymatic assay with peroxidase-labeled monoclonal anti-BrdU antibodies. HUVEC were preincubated for 30 min in PBS containing r(h) IL-1, r(h) IL-10 (R&D Systems) at 5, 10, 25, 50, 75 and 100 pg/mL or containing no r(h) IL-1 and no r(h) IL-10 (control).
Results: Mean value of absorbance for the *E. coli* strains was 0.45 ± 0.03. As compared to control values, bacterial adherence to HUVEC significantly increased following preincubation with r(h) IL-1 in the dose range of 5–100 pg/mL ($P < 0.05$). On the other hand, adherence of *E. coli* to HUVEC preincubated with IL-10 (dose range of 5–100 pg/mL) did not significantly differ from the control.
Conclusion: IL-1 may promote bacterial adherence to endothelial cells while IL-10 does not significantly affect the adherence.

P606 Perinatal *Listeria monocytogenes* strains from different phylogenetic lineages have the same virulence and induce identical inflammatory response by cord blood mononuclear cells

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Objectives: *Listeria monocytogenes* strains from phylogenetic lineage I are involved in quite 70% of human listeriosis whereas strains from lineage II are responsible for less than 30% and strains from lineage III for less than 1%. The aim of our study was to determine if only some strains belonging to lineages II and III are able to cause invasive disease in humans, whereas most lineage I strains can, or if all strains from lineages II and III are less pathogenic and are only able to infect the most immunocompromised people.
Methods: We compared the virulence of six *L. monocytogenes* perinatal strains, selected to be representative of the three phylogenetic lineages of the species. The virulence was evaluated by a plaque-forming assay with HT-29 cells. We also explored the immune response of cord blood mononuclear cells after 4, 8, 12, 18 and 24 h of incubation with heat-inactivated bacteria by measuring cytokine production. The TNF-alpha, IL-1beta, IL-6, and IL-8 production was evaluated using enzyme-linked immunosorbent assays.
Results: All of the strains were over the 3.34 cut-off (between 4.29 and 5.97 mean log) with the HT-29 model and can therefore be considered to be equally virulent. All strains induced the production of similar amounts of TNF-alpha and IL-1beta, and high concentrations of IL-6 and IL-8 were produced for the six strains. Thus, there is no difference between the strains from the three genetic lineages in terms of virulence or cytokine response.
Conclusions: Given the epidemiologic distribution of the serotypes responsible for human listeriosis and the genetic structure of the *L. monocytogenes* species we suggest that all strains from lineage I, a genetically homogeneous subpopulation, have a similar level of pathogenicity and may constitute a highly virulent group for humans. We also propose that lineage II, which is genetically more heterogeneous, is composed of strains with different levels of pathogenicity. The ones responsible for invasive diseases, particularly perinatal infections, display a similar level of pathogenicity to lineage I strains, whereas the other lineage II strains are probably less pathogenic for humans. Thus, the virulence of strains from lineage II seems to be strain-dependent.

P607 Study on interleukin-12 and interferon-gamma serum and stool levels of patients in the course of salmonellosis

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Objectives: To study interleukin-12 (IL-12) and interferon-gamma (IFN-gamma) serum and stool levels of patients in the course of salmonellosis and their correlation with the severity of the disease and bacteriologic sanation.
Methods: The study includes 37 patients with culture confirmed gastrointestinal salmonellosis. *Salmonella enteritidis* was the etiologic agent in 81%, *Salmonella typhimurium* in 13.51% and other *Salmonella* spp. were isolated in 5.49% of the cases. The patients were at the age of 18–57 years, with mild ($n = 12$), moderate ($n = 14$) and severe ($n = 11$) clinical variants of the disease. The control group comprised of 15 clinically healthy individuals at the same age. Serum concentrations of the examined cytokines were detected at the acute stage (2–4 days after admission) and reconvalescence, whereas the stool cytokine levels were determined only at the acute stage (concomitantly with the first sample). ELISA (Biosource, Belgium) was used for cytokine detection. Statistical methods involved nonparametric and Student *t*-tests.
Results: IL-12 and IFN-gamma serum levels were significantly elevated at the acute stage (Il-12: 319 ± 13; IFN-gamma: 1.87 ± 0.23) and at the reconvalescence as well (251 ± 9; 1.04 ± 0.19) in comparison with the healthy controls (IL-12 : 82 ± 12; IFN-gamma: 0.175 ± 0.02). The values were higher and more frequently increased in severe clinical variants and in patients with bacteriologic sanation. Stool concentrations of cytokines did not differ from these in healthy people.
Conclusion: Measurement of IL-12 and IFN-gamma serum levels in patients with salmonellosis is an important prognostic factor for the course and severity of the disease.

P608 Identification of G-CSF producing cells in *Salmonella* infection and endotoxin shock by in situ hybridization

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Objectives: The granulocyte colony-stimulating factor (G-CSF) is a prime hematopoetic growth factor and immunologic response modifier with a unique combination of antibacterial and anti-inflammatory effects. The recombinant protein is in broad clinical use to treat different forms of neutropenia and studies evaluating G-CSF for sepsis prophylaxis and for treatment of various infections are ongoing. In order to apply exogenous G-CSF efficiently, a better understanding of the endogenous formation is necessary. We focussed on the identification of producing cells in different tissues by detection of G-CSF mRNA levels via in situ hybridization (ISH).
Methods: Balb/C mice were challenged intraperitoneally with 3 mg/kg LPS for induction of endotoxin shock or with 1×10^7 CFU/kg *Salmonella typhimurium*. Tissue homogenates were controlled by G-CSF and β-actin specific real-time PCR for their G-CSF mRNA content. Failing with commercially available ISH kits, that provide short oligonucleotides (about 20 nt), we prepared a 360 nt RNA probe by molecular cloning, gel extraction and sequencing. Digoxigenin-labeling and labeling with S35 by in vitro translation was used for radiometric and antibody-based detection.
Results: Two and 4 h after challenge of the animals, an approximately 100–1000-fold increase of the quantity of G-CSF mRNA was observed by real-time PCR. The ISH carried out with synthetic oligonucleotides or Digoxigenin-labeled probes resulted in unspecific signals as evaluated by complementary probes and sham animal samples as control. Using radioactive ISH, the controls of all examined organs showed no signals. In the livers of challenged animals, single cells were identified by the accumulation of G-CSF ISH specific grains, located at the inner margin of blood vessels. Furthermore, stained cells were identified in the endocardium of the heart and kidney blood vessels. In the thymus, grained cells were located amidst the tissue at the border between cortex and medulla, as well as in the spleen at the border between red and white pulpa.

Conclusion: Based on these findings, identification of G-CSF mRNA-positive cells was achieved solely by the radiometric approach. Due to their location and their morphology these cells appear to be endothelial cells in the majority of tissues investigated. Not all endothelial cells were stained within a vessel, indicating a selective G-CSF formation in certain endothelial cells only.

P609 Analysis of CXCR1 and CXCR2 expression in premenopausal women with recurrent urinary tract infections

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Objectives: Polymorphonuclear neutrophils (PMN) play an important role in the host defence against urinary tract infections (UTI). PMN express two cell surface receptors (CXCR1 and CXCR2) for CXC chemokines, especially IL-8, which has a chemotactic effect on PMN. In animal models, it has been demonstrated that transepithelial PMN migration through the uroepithelium is mainly CXCR1 dependent. In this study we have investigated the existence of defects on the surface expression of CXCR1 and CXCR2 as well as of polymorphisms at the promoter and the coding region (exons 1 and 2) of CXCR1 in premenopausal women with recurrent urinary tract infection (RUTI) and in a control group.
Methods: Between January 2002 and January 2003 a case control study was performed. Cases were premenopausal women with RUTI defined as ≤ 3 UTI in the previous 12 months ($n = 20$). Controls were premenopausal women without previous UTI ($n = 30$). The CXCR1 and CXCR2 expression on PMN was measured by flow citometry using specific antibodies. Polymorphisms of a 519-bp region encompassing the exon 1 and the promoter region and in a fragment of 1203 bp encompassing the whole exon 2 of the CXCR1 were studied using a sequence based typing method with specific primers.
Results: Four out of the 20 cases (20%) had a decreased CXCR1 expression and 1 out of the 30 controls (3.3%) had it ($P = 0.14$). Two cases had a decreased CXCR2 expression (10%) and none of the controls had it ($P = 0.15$). Among the nine cases with previous UTI during childhood ($n = 9$) three (33.3%) had low expression of CXCR1 ($P < 0.05$) and two (22.2%) had low expression of CXCR2 ($P < 0.05$). No polymorphisms were detected neither in the promoter or in the exon 1 of CXCR1 while four polymorphisms were detected in the exon 2 with equal frequency in both groups.
Conclusions: The deficient CXCR1 and CXCR2 expression seems to play a pathogenic role in the UTI of premenopausic women having RUTI starting at childhood. The low expression of CXCR1 is not related with the existence of polymorphisms in the promoter, exon 1 and exon 2 region analyzed. Further studies are needed in order to address whether CXCR1 deficient expression is caused either by specific gene defects or by upstream regulatory mechanisms.

P610 Immunomodulatory effects of five *Bacteroides fragilis* strains differentiated by random primer PCR fingerprinting on the mouse macrophage cell-line IC-21

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Objective: To study the immunomodulatory effects of five different *B. fragilis* strains on the cytokine expression in macrophages.
Methods: Five *B. fragilis* strains were differentiated in two groups by random primer PCR fingerprinting. The resistance patterns of these strains against antimicrobial agents confirmed the differentiation in the two groups. Macrophages (IC-21 cell line) were incubated with either different amounts of *B. fragilis* alone or with both, *B. fragilis* and *Escherichia coli*-LPS. Negative controls were run with untreated cells, positive controls only with LPS. After 3 and 20 h of incubation, IL-1beta- and IL-6-mRNA were determined by qualitative PCR and semiquantitatively analyzed by HPLC. In addition, the synthesized cytokine-proteins were determined from supernatants by ELISA.
Results: Stimulation of macrophages with *E. coli*-LPS resulted in significantly augmented mRNA as well as cytokine product. The stimulation of macrophages with different amounts of *B. fragilis* resulted in minimally augmented cytokine levels compared to untreated cells. Co-stimulation of macrophages with *B. fragilis* and *E. coli*-LPS increased the levels of all cytokines. However, they did not always reach the levels of the positive control.
Conclusion: Our present study shows, that immunomodulatory effects of *B. fragilis* on cytokine expression in macrophages are involved in the pathophysiology of mixed aerobic/anaerobic infections.

P611 Variants of the R1 protein of group B streptococci

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Objectives: The R1 protein was expressed by approximately 10% of GBS (APMIS 109: 842, 2001). R1 belongs to a family of ladder-forming GBS proteins. Recently, the genes *alp2* and *alp3* encoding the proteins Alp2 and Alp3 were described (Proc Natl Acad Sci USA 97: 9630, 2000). Alp2 and Alp3 might be variants of the 'classical' R1 protein. We have approached this question by PCR and antibody-based methods
Methods: PCR which detects both the *alp2* and *alp3* genes or *alp2* and *alp3* separatedly (J Clin Microbiol 40: 620, 2002) were used. Rabbit antisera against the R1-expressing strains ATCC 12403 (*alp2* positive), JM9 and 64/95 (both *alp3* positive) were appropriately cross-absorbed to achieve specificity for R1 proteins and for each of the Alp2 and Alp3 proteins. Immunofluorescent antibody testing was used.
Results: Of 150 GBS strains of various capsular antigen types, 19 tested positive with the anti-R1 antibodies and these isolates were *alp2/alp3* PCR positive. Three out of 19 R1 positive strains expressed Alp2 and were *alp2* PCR positive, 16/19 expressed Alp3 and were *alp3* PCR positive. Alp2 or Alp3 expression was never found in *alp2/alp3* PCR negative GBS.
Conclusions: The findings substantiate the notion that Alp2 and Alp3 are variants of the classical R1 protein, and that these variants have common as well as variant-specific epitopes. Strains expressing Alp2 were capsular type III or type V isolates and all strains expressing Alp3 were type V isolates. These results are of importance both in the context of GBS serotyping and vaccine development.

P612 The effects of streptozotocin-induced diabetes on brucellosis of rats and interaction of insulin with *Brucella melitensis*

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Objectives: Depression of the natural defenses against infection in diabetics has long been suspected and investigated. This altered susceptibility to infection has been ascribed to disturbance in cellular innate immunity. On the other hand *Brucella* species and the disease spectrum are partially explained by the ability of the organism to evade host defense mechanism by virtue of its intracellular existence. The spectrum of disease depends on many factors including the immune status of host, the presence of other underlying diseases or conditions and the species of infecting organism. Thus, the aim of the present study was to investigate whether the streptozotocin-induced diabetes affects the course of *B. melitensis* infection in a rat model.
Methods: In the diabetic rat trial, diabetes was induced in overnight fasted rats by intraperitoneal injection of streptozotocin 4 days prior to the *B. melitensis* inoculation; 80 mg/kg STZ were administrated to the rats in group A and C for each of two consecutive days and 65 mg/kg STZ to rats in group B once. One hour prior to *B. melitensis* inoculation, 10 mU of NPH per g of body weight were injected intraperitoneally to the rats in group C for once.
Results: The mean of serum glucose level of diabetic rats are higher than rats in nondiabetic control group and statistically significant ($P < 0.05$, for each comparison) differences occurred in serum glucose level between groups A, B, C and control. The number of *B. melitensis* isolated (mean log10 CFU/organ weight) from spleen and liver of rats in group A, B and control was significantly ($P < 0.05$, for each comparison) different than each other. There was a slight decrease in the number of organisms recovered from spleen of rats in group C compared with those rats in Group A, which did not represent a significant ($P > 0.05$) difference. At a concentration of 10 U of insulin per ml, the growth rate of *B. melitensis* was not affected compared with that of cultures containing no insulin.
Conclusions: In this study, a model of *B. melitensis* infection was used in the setting of STZ-induced diabetes in rats. It was found that STZ injected rats exposed to *B. melitensis* infection had a significantly ($P < 0.05$) greater number

of *B. melitensis* in their spleen and liver than the rats in the control group. The reason for this might be related to disturbance in innate and adaptive immunity due to diabetic state induced by STZ injection.

P613 Bacteremia in patients with hidradenitis suppurativa undergoing surgical treatment with CO_2 laser stripping – secondary intention technique

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Introduction: Hidradenitis suppurativa (HS) is a cicatrizing and persistent disease of apocrine glandbearing areas in adults. The severity of the condition varies from a few suppurating lesions to widespread and disabling disease. The etiology is obscure, but suggested contributory factors include a genetic predisposition, comedones occluding the pilosebaceous apparatus, bacterial infection, and hormonal factors.
Objectives: The aim of the study was to investigate the amount and type of bacteria circulating in the bloodstream in patients with HS undergoing surgical treatment with CO_2 laser stripping – secondary intention technique.
Methods: Twenty-one patients (20 females and 1 male) were included in the study. One blood sample (8 mL) was taken before surgery, one during the operation and the last one 10 min after surgery. A group of five healthy individuals not undergoing any kind of operation was used as control. The blood was cultured by a lysis-filtration technique that earlier has been shown to be very sensitive. As the filter catches the micoorganisms and colonies are formed during the culturing the number of bacteria in the samples are easily determined.
Results: In six patients all samples were negative which indicates that the surgery method itself did not cause any spread of bacteria from the lesions. Bacterial growth in the first blood sample was found in nine patients, from the second sample in 10 and from the third sample in five patients. At least 10 different bacterial species were identified. The dominating bacteria were coagulase-negative Staphylococci (CNS), Propionibacterium sp., *P. acnes* and *S. aureus*. A few other anaerobes and some alfa-streptococci were also encountered. The bacterial findings in the blood samples are in agreement with the results from a previous study in patients with deep HS-lesions. In the five controls no microbial growth was detected.
Conclusion: The CO_2 laser stripping technique used did not cause any additional spread of bacteria in the blood stream. The evaluation of cultures containing microorganisms from normal skin flora is controversial. As the bacteria encountered in this study are in good agreement with findings in cultures from deep HS-lesions they seem to be relevant. The growth of bacteria in the first blood sample taken before surgery might indicate that some of these patients have bacteria continuously circulating in the blood.

P614 Evaluation of the OSIRIS system for disk diffusion susceptibility testing

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Objectives: OSIRIS is an automatic system for reading and interpreting disk diffusion susceptibility tests. The aim of our study was to evaluate the system by comparing the Osiris measured zone sizes to manually measured inhibition zones.
Methods: Six hundred and sixty strains were tested by the disk diffusion test, according to the NCCLS recommendations, using round 10 mm Mueller-Hinton agar plates. All strains were routine clinical isolates of diverse origins (urine, suppurations, feces, blood, bronchial aspiration) and included 486 enterobacteriaceae, 40 nonfermenters, 73 staphylococci and 61 enterococci. For each isolate 7–8 antimicrobials were tested: ampicillin, amoxicillin/clavulanic acid, ceftriaxone, ciprofloxacin, meropenem, tobramycin, oxacillin and cotrimoxazole. All zone diameters were read with OSIRIS and, if necessary, corrected on screen by the operator. Since the conventional manual reading is considered as the reference method, the inhibition zones were also measured with an electronic caliper using the mean of 3–4 measurements as the definite zone diameter.
Results: Essential agreement ($\leq$3 mm diameter differences) was obtained with 88% of the Gram negative strains (GNS). Ten percent of the GNS showed major errors (4–10 mm diameter differences) and 2% showed very major errors (>10 mm diameter differences). All GNS were easily read with OSIRIS, except some of the *Pseudomonas aeruginosa* strains. The results for ampicillin, amoxicillin-clav., meropenem, tobramycin and cotrimoxazole were excellent, showing essential agreement in more than 90% of the strains. The lower score for ciprofloxacin was due to the absence of a clear-cut inhibition zone and therefore a less accurate reading by OSIRIS. The scores for ceftriaxone improved significantly by changing the position of the disc on the plate. The results for staphylococci and enterococci were somewhat less satisfying, with essential agreement for 73 and 59% of the strains, major errors for 22 and 18% and very major errors for 5 and 23% of the strains, respectively.
Conclusion: The OSIRIS system is a useful method for the reading and interpretation of inhibition zone sizes in disk diffusion susceptibility testing. The position of antibiotic discs on the plate can improve reading performances. On screen adjustment, especially of major discrepancies, is useful to optimize the results but appears to be an easy task.

P615 High-level expression of the plasmid-encoded *AmpC* gene, blaCMY-7 does not compromise the virulent phenotype of a strain of *Salmonella typhimurium*

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Objectives: In a previous study with a *Salmonella typhimurium* (St) strain containing cloned *ampC-ampR* from *Enterobacter cloacae*, it was suggested that *ampC* beta-lactamase expression must be kept at low levels by AmpR to maintain a virulent phenotype. The purpose of this study was to determine how these findings obtained with a laboratory model system related to a virulent clinical isolate of St expressing the plasmid-encoded (PE) ampC blaCMY-7.
Methods: Disk induction assays were used to determine the induction potential of CMY-7. Conjugation was performed to transfer the plasmid encoding blaCMY-7 to *E. coli* J53AzR and St strain LT2. Five-hundred base pairs upstream of blaCMY-7 were generated for sequencing using Genome Walker, a PCR based methodology. The copy number of the plasmid encoding blaCMY-7 was determined using a comparative PCR-based method. Primer extension analyses were carried out to quantify blaCMY-7 RNA expression. Invasion potential was determined by in vitro invasion assays using gastric carcinoma cells.
Results: Conjugation and PCR results indicated that *blaCMY-7* was encoded on a large molecular weight plasmid that was present in two copies. Induction of AmpC b-lactamase activity was not observed using the disk induction assay. In addition, *ampR* was absent in a 500 bp region upstream of the *blaCMY-7* structural gene. The *blaCMY-7* expression was five-fold higher than *ampC* expression from a derepressed mutant of *Citrobacter freundii* (Cf). Comparisons between the clinical isolate, transconjugants of strain LT2, and strain LT2 without blaCMY-7 indicated that growth and virulence phenotypes were not compromised in St expressing *blaCMY-7*.
Conclusions: Contradicting a previous report, these data demonstrate that high-level expression of *ampC* in the absence of AmpR did not comprise the virulence phenotype of a clinical isolate of St. The origin of blaCMY-7 is the chromosome of Cf and derepressed mutants of Cf produce high levels of beta-lactamase leading to clinical resistance. However, expression of blaCMY-7 is 5-fold higher than a derepressed mutant suggesting that PE ampC beta-lactamases may be regulated differently in *Salmonella* isolates. Furthermore, these data suggest that plasmid copy number plays a minimal role in overall expression of blaCMY-7.

P616 Comparison of enterotoxin genes in *Staphylococcus aureus* strains from patients vs. healthy carriers

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Materials and methods: The presence of mecA-, enterotoxin (A–E and G–Q), TSST-1 genes was detected by the PCR technique in 161 strains of *Staphylococcus aureus* (SA). Strains ($n = 137$) were collected at Semmelweis University from throats, anterior nares and hands of healthy students, and at the Department of Pulmonology (DP) from sputum, throat, or bronchial lavage (BAL) of patients ($n = 24$). All isolates were identified by the classical methods. Antibiotic susceptibility was determined by microdilution (oxacillin), agar plate dilution (vancomycin) and disc diffusion (others). Phage types were determined by the standard international collection of phages.

Results: 87.5% (21) of the isolates from DP, and 74% (101) in the students' group harbored enterotoxin genes. In the 24 DP-strains SE-gene distribution was as follows: SEA 25% (6), SEB 16% (4), SEC 0%, SED 12.5% (3), SEE 0%, TSST-1 4.2% (1), SEG 62.5% (15), SEH 0%, SEI 62.5% (15), SEJ 12.5% (3), SEK 4.2% (1), SEL 0%, SEM 62.5% (15), SEN 66.7% (16), SEO 62.5% (15), SEP 25% (6), SEQ 12.5% (3). One strain was *mecA*-gene positive. In contrast, the 137 strains from students showed the following distribution: SEA 16% (22), SEB 11% (15), SEC 11.7% (16), SED 11% (15), SEE 1.5% (2), TSST-1 6.6% (9), SEG 34.3% (47), SEH 5.1% (7), SEI 38% (52), SEJ 11% (15), SEK 10.2% (14), SEL 11% (15), SEM 37.2% (51), SEN 38.7% (53), SEO 38% (52), SEP 5.8% (8), SEQ 16.1% (22). No *mecA*-gene was detected.
Conclusion: The overall number of SE-gene positive SA were (87 vs. 73.7%) in both groups high. Surprisingly, no MRSA was found in the students and only one in the patients. The most frequent SE-genes in the collection are SEG, SEI, SEM, SEN, SEO. The genes were mostly found combined and were present almost twice as frequent in patients' strains as in those of healthy carriers. The five-fold higher frequency of SEP in patients' isolates is noteworthy.
Acknowledgement: Supported by ÖAAD-MÖB.

P617 Virulence factors in *Serratia marcescens* hospital infections

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Objectives: The aim of this study was to analyze some of virulence factors of *S. marcescens* strains isolated in the Department of Medical Microbiology Ludwik Rydygier University School of Medical Science in Bydgoszcz over 6 years (1996–2002).
Methods: The collection of 136 *S. marcescens* strains was analyzed. Production of DNAse was examined on DNAse Test Agar, proteolytic properties – on Chemical Defined Medium (CDM) containing Casein Sodium Salt or Skim Milk Powder. Production of gelatinase was examined on solid medium with 3% gelatine. Hemolytic activity was determined on CDM containing 5% sheep erythrocytes and CDM with 5% human erythrocytes. Cell surface hydrophobicity (CSH) was analyzed using Salt Aggregation Test (SAT) and Bacterial Adhesion to Hydrocarbons (BATH) test. Adhesion of *S. marcescens* strains to natural human and sheep erythrocytes and tanned human and sheep erythrocytes was determined. Adhesion to polystyrene was evaluated with spectrophotometric method with usage of sterile, polystyrene, 96-well, round-bottomed tissue culture plates.
Results: All analyzed strains produced extracellular DNAse. Proteases were produced by 85.3–98.5% of *S. marcescens* strains. More than 70% of *S. marcescens* strains were CSH in both SAT and BATH tests. The highest percentage of strains (19.9%) adhered to tanned sheep erythrocytes while incubated in 37°C grades. Over 60% of analyzed strains adhered to polystyrene plates while incubated 24 h in 37°C grades.
Conclusions: *S. marcescens* strains are mostly isolated from hospital infections. Analyzed in this work virulence factors can help them to initiate infection process.

Pseudomonas aeruginosa

P618 Hemagglutination and adherence ability of *Pseudomonas aeruginosa* strains isolated from a variety of clinical sites

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Objectives: *Pseudomonas aeruginosa* is an opportunistic pathogen which rarely causes disease in healthy individuals and is a common human saprophyte. In most cases, the disease process begins with an alteration or a circumvention of normal host defenses. This may involve a disruption in the integrity of physical barriers to bacterial invasion such as the skin or mucous membranes, or their circumvention as in the case of intravenous lines, urinary catheters or endotracheal tubes. The ability of *P. aeruginosa* to adhere to the surface of mucous membranes of the human body or to medical devices is considered the initial step in its colonization and subsequent infection. In the present study, we compared the hemagglutination ability (HA) and adherence ability of wild-type *P. aeruginosa* strains to different cell lines and to polystyrene.
Methods: A total of 35 *P. aeruginosa* strain isolates from clinical specimens (urine, bronchial secretion, and ear, throat and wound swabs) were used. HA of strains was quantitatively determined in round-bottomed microtiter plates. The ability of strains to adhere to three different cell lines (Buffalo green monkey kidney, HeLa, and human fetal fibroblasts) was detected by immunofluorescence staining and the adherence of the strains to the wells of flat bottom polystyrene tissue culture plates was estimated by the spectrophotometric method.
Results: Most strains (89%) adhered well to the used cell lines, and there was no significant difference between adherence of the strains to the three cell lines used. The bacterial binding to the cells was almost completely blocked when bacteria were incubated at 80°C for 20 min before the adherence assay (one to five bacteria bound per 40 cells, compared with 30–110 bacteria per cell for the untreated bacteria, depending on strain). Fourteen of the 35 strains were positive for alginate production. The results of the spectrophotometric assay have revealed that the optical densities of formed biofilms of these 14 strains were 0.181–0.540. No statistically significant difference was observed in the adherence ability of isolates from different specimens to polystyrene. HA was detected in half of the strains and was not associated with alginate production.
Conclusions: Alginate is not involved in the hemagglutination ability and adherence ability of *P. aeruginosa* to the epithelial cell lines and fibroblasts.

P619 Effect of adjunctive clarithromycin on the treatment of foreign body-related *Pseudomonas aeruginosa* osteomyelitis model

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Backgraund: Biofilm formation may be the main factor of resistance to antibiotic therapy in treatment of implant-related infections in orthopedic surgery. Macrolide antibiotics can prevent the biofilm formation as shown in several in vitro studies. In this study, bacterial biofilm formation in foreign body-related *Pseudomonas aeruginosa* osteomyelitis model in rats, and the effect of clarithromycin, a macrolide, on the biofilm was investigated.
Methods: Right tibia of 26 rats were infected by *P. aeruginosa* resistant to clarithromycin but sensitive to ceftazidime. To verify osteomyelitis, radiographic examination was performed in all rats, and the tibia of three rats were removed, cultured, and then examined under electron microscope on the 14th day. The control group was formed by three infected rats that had no treatment. Ten rats had ceftazidime (1500 mg/kg/day) subcutaneously, and 10 rats had ceftazidime subcutaneously and clarithromycin (100 mg/kg/every 12 h) orally. After a treatment period of 20 days, the rat tibias and the foreign bodies were extracted under sterile conditions, cultured, and examined electron microscopically.
Results: The number of microorganisms grown on the bone tissue in the combined treatment group was significantly lower than the other groups ($P = 0.034$). However, there was no significant difference between the control and ceftazidim-only group ($P = 0.998$). The number of microorganisms grown on the foreign body in the only-ceftazidim treatment group was significantly higher than the combined treatment group ($P = 0.019$). Electron microscopic examination revealed that the bacterial concentration on the bone tissue in the group having combined therapy was clearly less than that on the other groups. The biofilm layer was eradicated, and the bacteria were

apparent in the group that had combined therapy; nevertheless, it was evident on the foreign body in the ceftazidime-only group.
Conclusion: Although clarithromycin was ineffective as a bactericidal against *Pseudomonas aeruginosa*, it enhanced the activity of concomitantly used bactericidal agents via preventing the formation of glycocalix structure on the surface of biomaterials.

P620 Treatment of experimental sepsis by multidrug-resistant *Pseudomonas aeruginosa* with the coadministration of antimicrobials, gamma-linolenic acid and arachidonic acid

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Objectives: Gamma linolenic acid (GLA) and arachidonic acid (AA) have been shown to render in vitro mulridrug resistant (MDR) *Pseudomonas aeruginosa* into susceptible to ceftazidime and amikacin (Giamarellos-Bourboulis *et al*. 2000, 44: 2187). The in vitro effect was tested in an experimental model of sepsis.
Methods: Sepsis was induced by the intravenous infusion of an 8.4 log10 inoculum of one MDR isolate of *P. aeruginosa* by a catheter inserted in the right jugular vein of 24 male rabbits. Thirty minutes later six animals were treated by the intravenous coadministration of 50 mg/kg of ceftazidime and 15 mg/kg of amikacin by a catheter in the left jugular vein. Six animals were treated by antimicrobials and an i.v. emulsion of 25 mg/kg of GLA infused within 10 minutes; six by antimicrobials and an i.v. emulsion of 25 mg/kg of AA infused within 10 minutes; six animals served as controls. Quantitative blood cultures were performed at regular time intervals. Animals were sacrificed 3.5 h after bacterial inoculation, and tissue samples of liver, spleen, lung and mesenterial lymph nodes (MLN) were homogenized and quantitatively cultured.
Results: Median log10 changes (CFU/mL) of bacterial blood counts of controls, of animals administered antimicrobials, of animals administered antimicrobials and AA, and of animals administered antimicrobials and GLA 30 min after treatment were: −0.52, −1.00, −1.30, and −2.00, respectively. Respective median values 90 min after treatment were: −1.26, −1.50, −2.04, and −1.97; 120 min after treatment: −0.95, −1.97, −2.04, and −1.96; and 180 min after treatment: −1.08, −2.19, −2.23, and −3.12. Respective median log10 values of bacterial counts (CFU/g) of liver at necropsy were 4.60, 5.47, 5.30, and 5.46; of spleen at necropsy 4.92, 4.90, 4.80, and 5.56; of the lower lung lobe at necropsy 4.49, 3.52, 2.44, and 3.22; and of MLN at necropsy 4.13, 2.47, 1.35, and 2.90.
Conclusions: The intravenous coadministration of an emulsion of AA and of GLA with ceftazidime and amikacin in experimental sepsis by MDR *P. aeruginosa* results in considerable enhancement of the antimicrobial effect of both antimicrobials in lowering blood and tissue bacterial counts. These results merit further clinical evaluation.

P621 Effective immunomodulatory treatment of experimental pyelonephritis by multidrug-resistant *Pseudomonas aeruginosa* with intravenous clarithromycin

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Objectives: Clarithromycin has been shown to inhibit in vitro biosynthesis of interleukin-8 at concentrations above 10 mg/L that are not achieved in serum after oral administration. The immunomodulatory effect of intravenously administered clarithromycin was assessed in experimental sepsis by multidrug resistant (MDR) *Pseudomonas aeruginosa*.
Methods: Sixteen male rabbits were applied: five controls (group A), five treated with amikacin (group B), and six treated with clarithromycin and amikacin (group C). Acute pyelonephritis was induced by ligation of the pelvoureteral junction of the right kidney and by bacterial inoculation in the right renal pelvis. An 8 log10 inoculum of one MDR isolate of *P. aeruginosa* was applied. MIC of amikacin was 512 mg/L and of clarithromycin 512 mg/L; no synergy between them was found after time-kill assay. Clarithromycin was administered at a dose of 80 mg/kg within 30 min by the vein of the left ear immediately after and 2 hours after bacterial inoculation. Amikacin was administered as a bolus by the same route at a dose of 15 mg/kg. Tumor necrosis factor-alpha (TNFα) was estimated in blood samples by the cytotoxic effect of serum on fibrosarcoma L929 cell line. Survival was assessed by Kaplan–Meier analysis.
Results: Mean ± SE of survival of group A was 3.00 ± 0.68 days, of group B 2.00 ± 0.29 days (pNS compared to group A) and of group C 9.75 ± 1.14 days ($P < 0.0001$ compared to both other groups). Median TNFα of serum of groups A, B, and C were 24.0, 26.0, and <11.5 pg/mL, respectively, 1 h after bacterial inoculation; 33.0, 35.0, and <11.5 pg/mL, respectively, 2 h after bacterial inoculation; 19.5, 25.0, and < 11.5 pg/mL, respectively, 24 h after bacterial inoculation; and 42.0, 55.0, and 24.0 pg/mL, respectively, 48 h after bacterial inoculation. Inoculated bacteria were cultured from the right kidney of all animals at necropsy.
Conclusions: The intravenous administration of clarithromycin decreases serum TNFα significantly and prolongs survival in experimental pyelonephritis by MDR *P. aeruginosa*. These results appear promising in clinical practice.

P622 Impact of gamma-linolenic acid and arachidonic acid on tumor necrosis factor-alpha in experimental sepsis by multidrug-resistant *Pseudomonas aeruginosa*

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Objectives: Polyunsaturated fatty acids like gammalinolenate (GLA) and arachidonate (AA) are considered as potent immunomodulators in chronic inflammatory states. Their effect was assessed in experimental sepsis by multidrug resistant (MDR) *P. aeruginosa*.
Methods: Sepsis was induced by the intravenous infusion of an 8.4 log10 inoculum of one MDR isolate of *P. aeruginosa* by a catheter inserted in the right jugular vein of 24 male rabbits. Thirty minutes later six animals were treated by the intravenous coadministration of 50 mg/kg of ceftazidime and 15 mg/kg of amikacin by a catheter in the left jugular vein. Six animals were treated by antimicrobials and an i.v. emulsion of 25 mg/kg of GLA infused within 10 min; six by antimicrobials, and an i.v. emulsion of 25 mg/kg of AA infused within 10 min; six animals served as controls. Systolic blood pressure and pulse rate were continuously recorded for 3.5 h by a catheter inserted in the right carotid and blood was sampled by a catheter in the left carotid. Tumor necrosis factor-alpha (TNFα) was assessed by the cytotoxic effect of serum on L929 fibrosarcoma cell line.
Results: Mean ± SE of TNFα before treatment was 163.2 ± 147.2 pg/mL in controls, 152.0 ± 75.5 pg/mL in animals administered antimicrobials, 36.0 ± 22.94 pg/mL in animals administered antimicrobials and AA and 23.17 ± 19.61 in animals administered antimicrobials and GLA. Respective values 30 min after treatment were 73.3 ± 41.9, 116.7 ± 99.7, 197.8 ± 101.5, and 30.0 ± 26.2 pg/mL; 90 min after treatment, 57.5 ± 34.7, 110.0 ± 101.8, 115.8 ± 98.7, and 146.0 ± 66.2 pg/mL; 120 min after treatment 62.8 ± 32.9, 110.2 ± 74.1, 94.7 ± 35.7, and 165.7 ± 81.7 pg/mL; and 180 min after treatment 168.0 ± 137.5, 121.0 ± 105.3, 63.2 ± 35.3, and 175.3 ± 103.9 pg/mL. Control animals and animals treated only with antimicrobials had hemodynamic instability with increased pulse rate and fluctuations in systolic pressure. Administration of GLA and AA resulted in stable values of systolic pressure and of pulse rate.
Conclusions: Intravenous administration of an emulsion of AA and of GLA with antimicrobials in experimental sepsis by MDR *P. aeruginosa* presents with hemodynamic benefit of the animals; AA seems to achieve a decrease of serum TNFα. These results merit further evaluation.

P623 Remodeling of sepsis by multidrug-resistant *Pseudomonas aeruginosa*. What does the experimental model propose as a target for immunomodulation?

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Objectives: To define the sequence of events leading to systemic inflammation in an experimental model of sepsis by multidrug-resistant (MDR) *Pseudomonas aeruginosa*.
Methods: Seventy-five male Wistar rats were used; 20 for assessment of survival and 55 for estimation of tumour necrosis factor-α (TNFα) and malondialdehyde (MDA). Sepsis was induced after the intraperitoneal

injection of an 8log10 inoculum of one MDR isolate of *P. aeruginosa*. Survival was estimated by Kaplan–Meier analysis. Animals were sacrificed in groups at regular time intervals, and blood was sampled by the abdominal aorta. TNFα was assessed by EIA and MDA by the thiobarbiturate assay.
Results: Mean ± SE of survival was 18.60 ± 1.84 h. All animals died after 36 h. Mean ± SE of TNFα 1, 2, 3, and 4 h after bacterial inoculation was 32.5 ± 28.8, 20.8 ± 6.3, 37.9 ± 9.6, and 50.5 ± 11.1 pg/mL (pNS compared to previous time intervals), respectively. Mean ± SE of MDA 1, 2, 3, and 4 h after bacterial inoculation was 0.74 ± 0.64, 0.40 ± 0.13, 0.81 ± 0.19, and 1.84 ± 0.35 M ($P < 0.001$ compared to previous time intervals), respectively.
Conclusions: In an experimental model of lethal sepsis by MDR *P. aeruginosa*, it is shown that increases of oxidant status, as assessed by estimation of MDA, occur earlier than the respective increases of TNFα. These results might create a new perspective for the application of antioxidants as immunomodulators in sepsis by MDR bacteria.

P624 Cystic fibrosis antibiotic susceptibility service in Scotland – 2002

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Objectives: Multi-resistant isolates from cystic fibrosis (CF) patients are referred to Aberdeen Royal Infirmary (ARI) from all over Scotland for extended susceptibility testing. This study describes patterns of antibiotic resistance in these isolates in the year 2002.
Methods: During 2002, 130 Gram-negative nonfermenting bacilli isolates from 62 different patients were submitted to ARI. MICs were established for each isolate against a range of antibiotics including amikacin (AMIK), gentamicin (GENT), netilmicin (NET), tobramycin (TOB), ciprofloxacin (CIP), levofloxacin (LEV), aztreonam (AZT), ceftazidime (CAZ), Piperacillin (PIP), Piperacillin/tazobactam (P/TAZ), imipenem (IMP), Meropenem (MER), colistin (COLIS), timentin (TIM), Cefpirome (CPIR), ampicillin/sulbactam (AMPSUL), chloramphenicol (CHLOR), minocycline (MINO), cotrimoxazole (COTRIM), and rifampicin (RIF). The *E*-test method was used.
Results: One hundred and thirty isolates were submitted from nine Scottish hospitals (range 2–46 isolates per hospital). The isolates were from 62 different patients whose age range was 9 months to 44 years. The 130 were broken down to 67 *Pseudomonas aeruginosa*, 42 *Burkholderia cepacia*, nine *Stenotrophomonas maltophilia*, seven *Pseudomonas* species and five *Alcaligenes* species. Up to 20 antibiotics were tested for each isolate, and a total of 3135 MICs were carried out. The MIC-90 for the majority of antibiotics tested was above the maximum value tested for each species. The MIC-90 fell within range for five antibiotics tested against *Pseudomonas* species; GENT (64 mg/L), NET (128 mg/L), TOB (8 mg/L), CIP (8 mg/L), COLIS (4 mg/L), for five antibiotics tested against *S. maltophilia*; LEV (6 mg/L), COLIS (24 mg/L), CHLOR (24 mg/L), MINO (3 mg/L), RIF (48 mg/L) and for only MINO (128 mg/L) when tested against *B. cepacia*.
Conclusions: Due to the highly resistant nature of these isolates from CF patients, single antibiotic therapy was rarely possible, necessitating the requirement for combination therapy.

P625 Investigation of resistance rates of *Pseudomonas aeruginosa* isolates to various antimicrobials

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Objectives: *Pseudomonas aeruginosa* is frequently isolated from nosocomial infections and has a complicated management as a result of high resistance rate. The resistance to antibiotics varies among hospitals and even clinics.
Materials and methods: The resistance rate of *Pseudomonas aeruginosa* strains isolated from 150 hospitalized or outpatients in Celal Bayar University Hospital between December 1999 and January 2002 to various antibiotics (ciprofloxacine, ofloxacine, norfloxacine, gentamicin, amikacin, netilmicin, amoxicillin-clavulanate, ceftazidime, ceftriaxone, cefepime, cefoperazon-sulbactam, imipenem, meropenem) were investigated. The isolated bacteria was identified by the usage of API 20 NE (Biomerieux) system. The susceptibility testing was performed by disk diffusion method (Oxoid) according to NCCLS criteria.
Results: The resistance rate of *Pseudomonas aeruginosa* strains were found as following; ciprofloxacine (8%), ofloxacine (26%), norfloxacine (10.7%), gentamicin (39.6%), amikacin (4%), netilmicin (5.3%), amoxicillin-clavulanate (96%), ceftazidime (13.3%), ceftriaxone (62.7), cefepime (2%), cefoperazon-sulbactam (7.3%), imipenem (10%), meropenem (2%). The resistance rate was found to be statistically higher in the strains which were isolated from hospitalized patients especially from the intensive care unit.
Conclusion: In order to control the nosocomial infections, the annual susceptibility rates of hospitals should be determined and effective antibiotic usage policy should be performed.

P626 In vitro synergism testing of antibiotic combinations and clinical outcomes of resistant *Pseudomonas aeruginosa*

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Objectives: This study was designed to determine whether synergism occurred between beta-lactams and aminoglycosides or fluoroquinolones for *Pseudomonas aeruginosa* isolates that were resistant to these antimicrobial agents and to evaluate clinical outcomes of infections by these isolates.
Methods: Using the chequerboard titration method, the activity in combination of beta-lactams, fluoroquinolones and aminoglycosides was investigated against 24 *P. aeruginosa* isolates resistant to these antibiotics, and the clinical records were reviewed to evaluate the clinical outcomes.
Results: Synergy was detected with one or more antimicrobial combinations against 15 of 24 (63%) isolates and partial synergy was detected with one or more combinations against all 24 isolates. Twelve of 24 (50%) isolates were considered pathogens of true infections. Only one of 12 infections was treated by antibiotic combination with synergism. Others were treated by antibiotic combinations without synergism. The rate of treatment failure was 8/12 (67%) in all of these infections.
Conclusions: The results of this study indicate that against *P. aeruginosa*, synergy may occur between beta-lactams, fluoroquinolones and aminoglycosides, although the strains are resistant to the individual antibiotics. The clinical outcomes of infections of resistant *P. aeruginosa* treated by antibiotic combinations without synergism are poor.

P627 Synergistic bactericidal activity of rifampin (RF) and colistin (CL) combination against multidrug-resistant *Pseudomonas aeruginosa*, in vitro and clinical study

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Background: MDR *P. aeruginosa* may be responsible for difficult-to-treat nosocomial infections and search for effective antimicrobial combinations is warranted. The addition of rifampin to colistin showed to be synergistic against some nonfermentative Gram-negative rods.
Study objective: To evaluate the activity of rifampin and colistin combination against MDR *P. aeruginosa* in vitro and in vivo.
Methods: Nine MDR *P. aeruginosa* clinical isolates were evaluated with the checkerboard method using the combination of RF and CL. Synergism was defined using the sum of fractional inhibitory concentration (S FIC): combinations with S FIC < 0.5 were considered to be fully synergistic, those with S FIC < 1 partially synergistic and those with FIC > 1 indifferent. Two strains were tested with the time-kill curve method to show bactericidal activity of this combination. Bactericidal activity of the antibiotic combination was defined as a ≥100-fold decrease in colony count by the combination compared with that by the most active single agent. Seven patients (pts) with deep-seated life-threatening MDR *P. aeruginosa* infections were treated with this antibiotic combination: two ICU pts with VAP, two pts with infected diabetic foot, one AIDS pt with sepsis, one lymphoma pt with pneumonia and sepsis, one pt with common variable immunodeficiency and pneumonia.
Results: All the tested strains resulted to be resistant to rifampin (MIC: 64 mg/L). The MIC in combination of rifampin decreased from four to six dilutions, reaching the range of susceptibility. The MIC in combination of colistin resulted to be in the range of susceptibility. The combination of rifampin and colistin was fully synergistic against four strains and partially synergistic against five tested strains. Bactericidal activity was showed against the strain tested with the time-kill curve method. All the seven patients treated with RF + CL combination were treated successfully.

Conclusions: The addition of rifampin to colistin show in vitro synergistic bactericidal activity against MDR *P. aeruginosa* strains. The activity of this combination in clinical practice seems to be effective also in difficult-to-treat severe MDR *P. aeruginosa* infections

P628 Effect of pyocin S2 produced by endemic *Pseudomonas aeruginosa* 42 A on the growth of human tumor and normal cell lines

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Objectives: Pyocins are bacteriocins produced by *Pseudomonas aeruginosa* which kill other strains from the same species. Pyocin S2 is a type of S pyocin produced by certain strains of *P. aeruginosa*, with molecular weight of 74 kDa. Pyocin S2 has potential DNases similar to the E2 group colicins, also inhibits lipid synthesis. This research investigates the cytotoxic effects of pyocin S2 produced by endemic *P. aeruginosa* 42 A on the growth of cultured human tumor and normal cell lines.

Methods: Pyocin S2 was produced by one of the endemic isolates *P. aeruginosa* 42 A after induced by mitomycin C (2 μg/mL). Purification of pyocin S2 was achieved by ion exchange chromatography using CM-Sepharose CL-6B and sodium phosphate buffer (pH 8) from an 80% ammonium sulfate precipitate of whole cell lysates. Pyocin activity of fractions were detected by using the Govan spot testing method. The purity of active fraction was confirmed by SDS–PAGE. Cytotoxic effects of pyocin S2 from endemic *P. aeruginosa* 42 A on HepG2 (Hepatocellular Carcinoma, Human), Im9 (Lymphoblast, Immunoglobulin Secreting, derived from Multiple Myeloma, Human) and HFFF (Human Fetal Foreskin Fibroblast) cell lines were studied by MTT assay method. HepG2 and HFFF grew as monolayer, whereas Im9 grew in suspension.
Results: Our results showed that pyocin S2 from *P. aeruginosa* 42 A exhibited inhibitory effects on the growth of tumor cell lines HepG2 and Im9 after 5 days incubation. No inhibitory effect was observed on the growth of normal cell line HFFF.
Conclusions: In summary, the observation presented here point to the potentialities inherent in pyocin S2 as a diagnostic tool for sensitive cancer cells suggesting that these molecules may have therapeutic value as nontoxic inhibitors of some neoplasia.

Diagnosis of mycobacteria

P629 Improvement of the PCR diagnostic method in patients with kidney tuberculosis suspicion

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Introduction: Kidney tuberculosis is an important clinical problem, especially because of the difficulties in a diagnostic examination. While nonspecific clinical presentation and variable radiographic appearance often mimic other pathologic lesions, limited bacterium discharge to urine makes it difficult to isolate *Mycobacterium tuberculosis* from the urine samples.
Objectives: The aim of our study was to indicate the high value of nested PCR method in case of kidney tuberculosis suspicion and limited mycobacterium discharge in urine. We also emphasized the role of blood PCR in these patients. Since the proinflammatory cytokines TNF-alpha, INF-gamma, and IL-12 are regarded to be significant diagnostic markers in many infectious diseases, we showed the importance of these immunologic indicators in diagnosis of the extrapulmonary tuberculosis.
Materials: Thirty patients attending the urology clinic with suspicious of kidney tuberculosis were evaluated. The main symptoms suggestive of kidney tuberculosis were: significant changes in urine and urographic image, weight loss, and weakness. The PCR for *M. tuberculosis* was done for both blood and urine samples obtained from all patients. The blood levels of TNF-alpha, INF-gamma and IL-12 were quantified by ELISA test and compared with control group.
Results: In 12 patients, presence of *M. tuberculosis* was detected neither in urine nor in blood samples. In 11 patients, the product of gene amplification was noticeable only in urine samples, while in seven patients it was present in both urine and blood samples. Additionally, the differences in serum cytokine levels between patients with kidney tuberculosis and healthy people were found. TNF-alpha concentration was about twofold higher in the positive patients; IL-12 concentration was about fourfold higher and the differences between IL-12 levels were statistically important ($P < 0.05$). However, no significant differences were found in INF-gamma level among all groups.
Conclusions: Owing to high sensitivity, the nested PCR is particularly useful in paucibaciliary situations such as kidney tuberculosis. Next to urine PCR, PCR for blood specimen is also an important diagnostic marker during the examination for the disease. Our study also indicates that alterations in certain cytokines levels may be helpful in differentiating the lesion.

P630 Early diagnosis of tuberculous meningitis by polymerase chain reaction

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Introduction: Due to inconsistent clinical findings, rarity, and the lack of a rapid, sensitive, and specific test, tuberculous meningitis (TBM) is difficult to diagnose, although the rapid diagnosis is of vital importance for patients. The aim of this study was the evaluation and the contribution of PCR in the early and specific diagnosis of TBM.
Materials and methods: A total of 23 cerebrospinal fluid (CSF) samples from patients with symptoms suggestive of TBM were studied. The pellet of the centrifuged CSF samples was examined for mycobacteria using Ziehl–Neelsen (ZN) staining, and it was cultured onto Lowenstein–Jensen slope. Mycobacterial DNAs from the samples were amplified using an IS6110 gene-specific PCR (fragment 123 bp). In order to evaluate the specificity of PCR in the diagnosis of TBM, seven CSF samples from patients with microbial meningitis (*Streptococcus pneumoniae, Neisseria meningitidis*, *Cryptococcus neoformans*, *Brucella melitensis*) were examined by PCR. A strain of *Mycobacterium tuberculosis* isolated from a CSF sample was used as a positive-control.
Results: All the 23 CSF samples were negative by ZN staining. Seven culture-positive samples for *M. tuberculosis* were also positive by PCR. The culture was negative in all the remaining 16 samples, while the PCR was positive in nine and negative in seven samples. The PCR confirmed the diagnosis of TBM in 16 patients while the CSF culture only in seven patients. The samples from the microbial meningitis cases were all negative by PCR.
Conclusion: The CSF PCR is a rapid, sensitive, and specific diagnostic test that contributes to the early diagnosis of TBM.

P631 A 4-year experience of Cobas Amplicor system use for the rapid detection of *Mycobacterium tuberculosis* complex in respiratory and nonrespiratory specimens in Greece

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Objectives and methods: To demonstrate the experience of a clinical microbiology laboratory on DNA amplification assay for routine detection of *Mycobacterium tuberculosis*, we performed the Cobas Amplicor MTB-PCR test (Roche Diagnostics) in 7722 pulmonary and 1451 extrapulmonary specimens collected from 3321 patients. The results were compared with those of the conventional Lowenstein–Jensen medium, MB/BacT liquid culture, and the clinical findings.
Results: Among the 254 culture-positive MTB respiratory specimens, 240 were positive with Cobas Amplicor MTB-PCR test, and among the 7300 culture-negative specimens, 45 (0.6%) were positive by the PCR. After detailed interpretation, the overall sensitivity, specificity, positive and negative predictive values for the Cobas Amplicor MTB-PCR test were 84.5, 99.8, 94.1, and 99.4%, respectively. The Cobas Amplicor MTB-PCR assay was more sensitive for smear-positive respiratory specimens, exhibiting sensitivities of 97.1 for smear-positive and 48.6% for smear-negative specimens. For the 18 culture-positive MTB (smear-negative) nonrespiratory specimens, nine were positive by the PCR. None of the 1384 culture-negative nonrespiratory specimens was positive by the PCR. So, the overall sensitivity,

specificity, positive and negative predictive values for the Cobas Amplicor MTB PCR test were 50, 100, 100, and 99.4%, respectively. The inhibition rates detected by the internal control of the test were 2.2 for respiratory and 3.4% for nonrespiratory specimens.
Conclusion: Cobas Amplicor MTB-PCR test might enable clinical microbiology laboratories with considerable previous experience in molecular biology testing to perform PCR and confirm TB infection immediately, leading to a better clinical management.

P632 Evaluation of the routine use of MTD (Gen-Probe) for the direct detection of *Mycobacterium tuberculosis* complex in clinical specimens

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Objectives: Evaluation of sensitivity and specificity of the molecular detection of *Mycobacterium tuberculosis* complex by MTD (Gen-Probe, Inc. San Diego, CA) for diagnosis of pulmonary and extrapulmonary tuberculosis in comparison with culture results and clinical outcome.
Methods: We studied 2094 specimens from 1615 patients submitted to the microbiology lab of our hospital over a 3-year period (2000–2002). Forty-three percent of the samples were pulmonary (sputum, BALs, gastric lavage, bronchial washing), 49% body fluids and aspirates (CSF, pleural effusions, pericardial effusions, ascitic fluids, urine, others), and 8% blood and bone marrow aspirates. The interpretation of the MTD results was done considering values <30 000 RLU as negative results according to the manufacturer. Results of 30 000–300 000 were considered as indeterminate. All the MTD results were compared with BACTEC culture (BACTEC9000MB, Becton Dickinson) and culture on LJ medium, and resolution of discordant results was accomplished by incorporating clinical data and repeated specimen analysis.
Results: After resolution of discrepant results and repeated testing of the specimens with indeterminate results (>30 000–300 000 RLU), we had total 82 positive MTD samples. Among the 48 of them with results above 300 000, 42 samples were confirmed as positive by culture or clinical outcome. Of the other 34 positive MTD samples with results in the zone 30 000–300 000, only 11 had clinical confirmation. The sensitivity, specificity and positive predictive value for respiratory specimens ($n = 39$) were 100, 99, and 80%, respectively. With body fluids and other aspirates ($n = 23$), those values were 100, 99, and 74%, respectively, when for blood and bone marrow aspirates ($n = 20$) they were 100, 91, and 25%. By implementing an equivocal zone and considering all the samples with results <300 000 as negatives, the overall specificity and positive predictive values were improved (from 98.5 and 65% to 99.7 and 88%, respectively).
Conclusions:

1. Inclusion of MTD test in the routine testing algorithm of a diagnostic laboratory when testing samples are appropriate selected, can result in the rapid, reliable, and accurate detection of MTB in clinical specimens.
2. The number of false positive results can be limited by implementing an equivocal zone.
3. Regarding the blood specimens, we propose the reconsideration of the positive cut-off value.
4. Amplification assays cannot still replace the conventional diagnostic techniques.

P633 Rapid identification of mycobacterial isolates by PCR-restriction fragment length polymorphism analysis (PCR-RFLP) of *rpoB* gene

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Objective: The purpose of this study was to evaluate the PCR-RFLP analysis of *rpoB* gene for the rapid identification of mycobacterial isolates.
Methods: Three hundred and forty-one clinical isolates (250 *Mycobacterium tuberculosis*, 32 *M. gordonae*, 28 *M. fortuitum*, 9 *M. chelonae*, 9 *M. avium*, 6 *M. intacellulare*, 5 *M. kansasii*, and 2 *M. malmoense*) included in this study were cultured in a liquid medium (Bactec MGIT 960 system, BBL, Becton Dickinson Microbiology Systems) and on Lowenstein–Jensen medium. Bacterial DNA was prepared as described by Sion et al. (Sion et al. 1999, Eur. J. Clin. Microbiol. Infect. Dis. 18, 346–351) and a segment of the RNA polymerase gene (*rpoB*) was amplified as described by Kim et al. (Kim et al. 2001, J. Clin. Microbiol. 39, 2102–2109). The amplified PCR products were digested with four restriction enzymes (*Hin*d II, *Hae*III, *Mva*I, and *Aca*II), and the mixtures were electrophoresed on a 3% Metaphor agarose. The isolates were identified using the PCR-RFLP algorithm (Kim et al. 2001, J. Clin. Microbiol. 39, 2102–2109), and the results of identification were compared with those obtained by conventional biochemical and AccuProbe tests.
Results: The results of the PCR-RFLP analysis of *rpoB* gene were in excellent concordance with that obtained by routine biochemical identification tests. All the 341 mycobacterial isolates included in this study have been correctly identified. On the other hand, AccuProbe identified 302 of the 341 isolates (90%) (250 *M. tuberculosis*, 32 *M. gordonae*, nine *M. avium*, six *M. intacellulare*, and five *M. kansasii* isolates) but failed to identify the remaining 39 non-tuberculous mycobacterial isolates (28 *M. fortuitum*, nine *M. chelonae*, two *M. malmoense*).
Conclusions: The PCR-RFLP method is a rapid and a reliable method for the identification of mycobacterial isolates. It is faster than the conventional methods, technically simple, less expensive, and can identify more mycobacterial species than AccuProbe tests. Therefore, we recommend the use of this method for routine identification of mycobacterial isolates in clinical laboratories.

P634 Evaluation of the VIDAS PROBE MTB test: analytical sensitivity, interference, and specimen-processing robustness

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Objectives: To evaluate the analytical sensitivity of the VIDAS PROBE[1] *Mycobacterium tuberculosis* (MTB) Test (BioMerieux, Inc., Rockland, MA). Additionally, interference by endogenous and exogenous substances and the robustness of the MTB specimen processing procedure to inactivate *Mycobacterium* was assessed.
Methods: Thirty-one *M. tuberculosis* (MTB) strains, both sensitive and resistant to current antibiotic therapies, were tested with the MTB assay in both pure system and sediment matrix to evaluate sensitivity. Mucin, whole blood (WB), white blood cells (WBC), albuterol, saline, and epinephrine were tested in the MTB assay at varied concentrations in combination with MTB at 16 CFU/test to evaluate interference. The robustness of the MTB specimen-processing procedure to inactivate *Mycobacterium* was performed by varying the time and temperature of the heat step and the time of the sonication step while processing *M. tuberculosis* and *M. gordonnae*. The processed lysates were then cultured to determine if viable organisms were recovered.
Results: All 31 MTB strains were detected at a level of 0.5 CFU/test in both pure system and sediment matrix. No interference was observed with mucin and whole blood when tested at levels equal to or less than 10%. No interference was observed with WBCs at concentrations equal to or less than 80%. Exogenous substances did not interfere when tested at the following concentrations: equal to or less than 20% with albuterol and saline, and equal to or less than 8% epinephrine. No viable *Mycobacterium* was recovered from lysates when the temperature of the heat step was varied from 80 to 95°C in combination with a 15 min sonication step or when the duration of the 95°C heat step was varied from 10 to 20 min in combination with 0, 10, 15, and 20 min sonication. Growth was observed when the heat step was omitted, regardless of sonication time from 10 to 20 min.
Conclusion: The MTB test is a sensitive method that is not influenced by endogenous or exogenous substances at concentrations normally found in respiratory specimens. This study clearly demonstrated the robustness of the MTB specimen processing procedure to inactivate *Mycobacterium* while showing the 95°C heat step to be the critical parameter for effective nactivation.

[1]This device has not been approved by the US FDA and is not commercially available.

Diagnosis of tuberculosis

P635 PCR for nasal swabs from pulmonary tuberculosis patients

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Objectives: To investigate the presence of tubercle bacilli on nasal mucosa of pulmonary tuberculosis patients using polymerase chain reaction (PCR).
Methods: A group of 31 smear-positive pulmonary tuberculosis patients were entered into the study, and PCR were performed for nasal swabs from them. Swabs dipped in sputum samples from the same smear-positive patients were used as positive controls. The method of Boom et al was used for DNA extraction and a simple PCR for *Mycobacterium tuberculosis* of Eisenach et al was carried out to detect specific IS6110 DNA sequences using a set of primers with a detection limit of fewer than 10 bacilli.
Results: Tubercle bacilli were detected in nasal swabs from 13/31 smear-positive tuberculosis patients (42%). All of the sputum swabs were positive for *M. tuberculosis*.
Conclusions: The results of this investigation reveal that *M. tuberculosis* can be found in the noses of some pulmonary tuberculosis patients. It is suggested that more studies should be done on this matter to render more information on the relation between nasal carriage of tubercle bacilli and pulmonary tuberculosis.

P636 Direct detection of resistance in *Mycobacterium tuberculosis* from clinical samples by real-time PCR

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Objectives: In recent years, several real-time PCR designs have been developed to detect resistance in *Mycobacterium tuberculosis* (MTB) from cultured samples. Nevertheless, little effort has been made to detect resistance directly from clinical samples. Our aim is to adapt a real-time PCR design, recently developed in our laboratory, to detect resistances against rifampin and isoniazid directly from clinical samples.
Methods: Clinical samples from patients with phenotypically resistant isolates of MTB were retrospectively recovered from our collection. Twelve clinical samples (11 respiratory and one lymph node, 11 acid-fast positive) were available for study. Of these, five were isoniazid-resistant, three rifampin-resistant and four multiresistant. Thirteen samples with susceptible MTB were also selected as controls. Resistance to rifampin and isoniazid was detected by Light-cycler technology using fluorescent probes (FRET) designed to detect all mutations to rifampin and those most frequent for isoniazid in MTB.
Results: All but one of the resistant samples with mutations confirmed by DNA-sequencing were assigned as resistant by real-time PCR. For the remaining resistant samples, no amplification was obtained by real-time PCR. All samples with susceptible MTB were correctly assigned by real-time PCR. The specificity of the assay was proved by assaying different respiratory pathogens (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycobacterium avium*, *Enterococcus faecalis*, *Escherichia coli*, *Streptococcus aureus*, *Nocardia asteroides* and *Pseudomonas aeruginosa*) and no fluorescent signal was obtained in these cases.
Conclusions: Our design can rapidly (60 min) detect resistance against rifampin and isoniazid directly from clinical samples. This method can contribute significantly to optimising the treatment of tuberculosis at diagnosis, and it also enables us to rapidly detect resistant strains, thus limiting their transmission. Additional efforts should be made to adapt this design to acid-fast-negative samples.

P637 Ziehl–Neelsen staining and PCR study in paraffin-embedded slides of tuberculous granulomas

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Objectives: In the diagnosis of tuberculosis (TB), the use of methods studying acid-fast bacilli (AFB) and *Mycobacterium tuberculosis* complex DNA in biopsy materials is also recommended. In this study, we aimed to find the sensitivity of these diagnostic methods.
Methods: Forty-five paraffin-embedded granulomatous samples taken from patients with TB were stained with Ziehl–Neelsen by a pathologist in order to detect AFB. Thirty-eight out of the samples were also studied by polymerase chain reaction (PCR) to detect *M. tuberculosis* complex DNA. IS 6110-specific primers, which could amplify all *M. tuberculosis* complex strains, were used for amplification.
Results: While AFB were not seen in any (0/45), *M. tuberculosis* complex DNA was detected in 14 out of 38 (37%) samples. AFB have been seen in tuberculous granulomas in 0 to 35% in previous studies. The sensitivity of PCR to detect *M. tuberculosis* complex DNA (86–88%) was reported to be higher than that of the Ziehl–Neelsen staining to detect AFB.
Conclusion: While we could not detect AFB by Ziehl–Neelsen staining in paraffin-embedded granulomatous TB tissues, PCR was positive in nearly one-third of the same tissues.

P638 Investigation of genetic heterogeneity in insertion sequence IS6110 among *Mycobacterium tuberculosis* isolates from pulmonary and extrapulmonary patients using DNA fingerprinting for epidemiological purposes

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DNA fingerprinting of *Mycobacterium tuberculosis* has been shown to be a powerful epidemiologic tool. Restriction enzyme analysis is a fingerprinting technique which is widely used to investigate molecular epidemiology of *M. tuberculosis* and other related mycobacteria. A total of 150 clinical isolates of *M. tuberculosis* were collected from TB reference unit, PHLS, Ahwaz, Iran. The PCR-REA employed uses a simple DNA extraction followed by a PCR step involving primers based on the insertion sequence IS6110. Restriction enzyme analysis was then performed on the amplification products using *Hae*III enzyme. IS6110-REA produced five clusters that accounted for 78% of the low-fragment number isolates (117 patients) and two clusters for 22% of the high-fragment number isolates (33 patients). All the isolates from extra-pulmonary cases had identical restriction patterns, and prisoners (three patients) had unique pattern too. Patients within two of the clusters were found to be epidemiologically related, while no relation was observed in patients in the other clusters. In conclusion, PCR-REA is rapid, reproducible and as discriminating as IS6110-RFLP analysis to investigate the *M. tuberculosis* epidemic and study of genetic heterogeneity among *M. tuberculosis* strains.

P639 New perspectives in the diagnosis of lung tuberculosis

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Objectives: The aim of this study was to investigate the combined applications of nuclear acid amplification techniques in the detection of *Mycobacterium tuberculosis* (MTB) and criteria proposed by the Belgium Centres for Molecular Diagnosis (CMD).
Methods: During the study period, direct examination (DE), mycobacterial culture, and PCR were performed on 477 respiratory samples of 262 patients older than 16 years of age. The results were reviewed retrospectively for over a period of 2 years (May 2000–May 2002). The CDM proposed to allow a DNA amplification only if following criteria are met: (i) the patient may not have had an anti-MTB treatment during the last 12 months; (ii) a positive DE, or in case of a negative DE; (iii) there must be clinical signs of infection and a positive radiography (RX or CT) and no other infectious agent may be found.
Results: Among the 262 patients, 85 presented a positive culture, giving a recovery rate of 32%. The patients could be divided into three groups: DE-positive group, DE-negative group complying the criteria, and DE-negative group with drawbacks in one or more criteria. In the 46 patients with a positive DE, the sensitivity of the PCR was 95%. One hundred and fifty-nine of the 216 patients with a negative DE met the inclusion criteria. Thirty of those had a positive culture for MTB (on a first sample), which were confirmed by PCR in 20 cases (60.6%). If we used two samples per patient,

37 patients had a positive culture confirmed by PCR in 32 of those (86.5%). The inclusion criteria were not met by 57 of the patients with a negative DE. No positive culture was found, and PCR gave two false-positive results. 46% of the PCR could be avoided if we looked for the results of routine cultures made 5 days prior till 2 days after the analysis. If we also take the radiography in consideration, we could avoid 98% of unnecessary DNA amplification tests.
Conclusion: The results of our study plead for a new protocol for the detection of MTB in patients suspicious for lung tuberculosis: (i) in a positive DE sample, a nucleic acid amplification can be used to exclude an atypical mycobacterium; (ii) when the DE is negative, we will wait for the results of routine cultures (5 days prior to 2 days after analysis), prior to utilisation of expensive nucleic acid amplification tests.

P640 Value of gastric lavage in diagnosis of pulmonary tuberculosis

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Objective: The aim of this study was to find value of gastric lavage in the diagnosis of pulmonary tuberculosis.
Methods: From May 1994 to February 1999, 886 gastric lavage were sent from hospitals of Umia city to heath reference tuberculosis laboratory. Early morning gastric lavage was collected. All specimens decontaminated and processed according to Petrrofs method. The supernatant was removed, and sediment were subjected to smear examination for acid-fast bacilli and culture.
Results: Of 886 patients whose gastric lavage had been sent to health reference laboratory, 54 (6.3%) had positive results for *Mycobacterim tuberculosis*. The mean age of patients was 36 years (SD ±21 years), and 20 (37%) patients were males and 34 (63%) were females. Of 54 patients, 33 had positive results for MTB, both direct smear and culture. In 10 patients, the result of direct smear was positive but the result of culture was negative; on contrary, 12 patients were positive for direct acid-fast smear but the results of the culture were negative. We expected that all positive direct smears had positive culture results because the sensitivity of culture in comparison of direct smear is very high. After investigation, we found that there was a long time delay between collection of gastric lavage and sending of specimens to laboratory and there was not any evidence of neutralization gastric aspirate by sodium bicarbonate after collection.
Conclusion: It is concluded although gastric lavage is a valuable specimen for diagnosis of tuberculosis but delay in sending of specimen to laboratory yields low rate of isolation MTB as, the aspirate contains hydrochloric acid from the stomach, which may kill MTB.

P641 Evaluation of the BACTEC 960 System and conventional culture media for recovery of mycobacteria from different clinical specimens

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Objectives: A total of 3242 clinical specimens were studied in 22-month period. BACTEC MGIT 960 system (BD, Sparks MD) is an automated growth and detection system using an oxygen sensitive fluorescent indicator to monitor microbial respiration and automatically determines and reports results. We compared the BACTEC MGIT 960 system with conventional Löwenstein-Jensen (LJ) medium for detection and recovary of mycobacteria from clinical specimens.
Methods: We performed standard N-acetyl-L-cysteine-2% NaOH procedure for decontamination for both BACTEC and solid media culture. BACTEC MGIT 960 media were supplemented with the antibiotic mixture polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin (PANTA) and growth supplement (Becton Dickinson). Solid media were incubated at 37°C for 8 weeks and were inspected weekly or until mycobacterial colonies were seen.
Results: We isolated 162 (4.52%) *Mycobacterium tuberculosis* and 10 (0.3%) non-tuberculosis mycobacteria. One hundred and six, 27, 14, eight, two, four and one of *M. tuberculosis* strains were isolated from respiratory system, abscess, body fluid, urine, thorax tube, biopsy, and stool specimens, respectively. Eight, one, one of non-tuberculosis mycobacteria were isolated from respiratory system, gastric lavage, and urine, respectively. BACTEC MGIT 960 detected 162 (100%) of total *M. tuberculosis* and 10 (100%) of non-tuberculosis mycobacteria. Solid media (LJ) detected 160 (98.7) of *M. tuberculosis* and 10 (100%) of non-tuberculosis mycobacteria. Ninety (55.5%) specimens were found to be positive by acid-fast stain method. Only, one lymph node was found to be positive in acid-fast stain. We did not recover it both in BACTEC MGIT 960 system and solid media. We found almost no difference between MGIT 960 SYSTEM and solid media for detection of mycobateria, but we found a big and meaning difference for recovery times of mycobacteria. Average time needed for detection of mycobacteria was 10.5 days in BACTEC MGIT 960 system but it was longer in solid medium (26.5 days). We also used antimicrobial susceptibility testing (AST) OF BACTEC MGIT 960 system. We had the test results in average of 7 days. The time needed for susceptibility tests in solid media was 20 days or more.
Conclusions: As conclusion, We had the shortest times to detection and susceptibility test results of mycobacteria with BACTEC MGIT 960 system when compared with solid media for the avarege times to detection of mycobacteria.

P642 Evaluation of the LiPA assay in clinical *Mycobacterium tuberculosis* specimens

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Introduction: In controlling the spread of tuberculosis (TB), rapid diagnosis of *Mycobacterium tuberculosis* complex (MTC) and identification of resistance to rifampin (RIF), one of the most potent antituberculosis drugs, are crucial for efficient treatment and control of multidrug-resistant strains. The LiPA RIF. TB assay (Innogenetics, Belgium) provides direct detection of MTC and the determination of RIF resistance in respiratory specimens.
Objectives: We evaluated the performance of the LiPA RIF. TB assay in clinical respiratory samples vs. traditional methods and the BACTET MGIT 960 system (Becton Dickinson, USA). Cultures of the samples which resulted negative to LiPA RIF. TB were assessed by LiPA Mycobacteria for the identification of MOTT.
Methods: Between May and October 2002, 135 clinical respiratory specimens were collected from patients suspected of TB and recovered at the A.O. 'E. MORELLI' of Sondalo (northern Italy), and examinated by fluorescence microscopy for acid-fast bacilli, solid (Lowenstein–Jensen, Difco Laboratories, USA) medium and liquid culture medium (BACTET MGIT 960). A portion of all the decontaminated samples was sent to our laboratory for the LiPA evaluation.
Results: Among the 135 respiratory specimens, MTB bacteria were identified by LiPA RIF. TB assay in 98 samples, of which 58 were positive by both microscopy and LiPA assay. Ten of 98 samples were RIF resistant. Mutations at codon 531 were the most prevalent (8 of 10 or 80%). LiPA RIF. TB assay showed perfect concordance with the Bactet MGIT 960 system with the potential to reduce time of determination of RIF resistance in a range of 8–25 days. Among the 37 specimens which resulted negative to LiPA RIF TB assay, 16 were MOTT and were correctly identified by LiPA Mycobacteria. Twenty-one of 37 samples which resulted negative to LiPA assay were negative with microscopy and cultures.
Conclusions: The LiPA RIF TB assay offers a rapid and reliable molecular approach to the detection of *M. tuberculosis* and determination of RIF resistance in addition to nonmolecular systems, especially in the management of patients with active disease.

P643 Comparison of BACTEC MGIT 960 and BACTEC 460 TB for detection of mycobacteria: a meta-analysis

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Objectives: A combination of solid and liquid media is currently recognized as the procedure of choice for primary isolation of mycobacteria from clinical specimens. Recently, several nonradiometric methods have been introduced, including the BACTEC MGIT 960 system (B960). In order to obtain an overview of the diagnostic accuracy of B960 for the detection of mycobacteria, a systematic review and meta-analysis of studies reporting the comparative specificity and sensitivity of BACTEC 460 TB system (B460) + solid media (the 'gold standard') and B960 was performed.
Methods: An electronic and hand search was performed. Studies were included in the analysis if the comparison between tests was performed prospectively in a consecutive series of patients. We calculated summary receiving operating characteristic curve, diagnostic odds ratio, pooled sensitivity, and specificity.

Results: In the 10 identified studies, a total of 1292 mycobacteria were recovered from 15 837 clinical specimens. The B960 had a sensitivity of 82.3% (95% CI, 80.1–84.4) and a specificity of 99.5% (95% CI, 99.4–99.6). B460 had sensitivity of 84.0% (95% CI, 81.9–86.0) and a specificity of 100% (95% CI, 99.9–100). Specificity, but not sensitivity, was significantly higher with the B460 ($P < 0.001$). Time to detection of mycobacteria was significantly shorter with the B960 compared with the B460 (13.0 days vs. 15.2 days, $P = 0.02$).
Conclusion: Compared to the well established B460, the B960 has similar sensitivity, lower specificity, but higher rapidity in detecting mycobacteria.

P644 Evaluation of BioFM liquid medium for recovery of *Mycobacterium* species from clinical specimens

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Objectives: BioFM (Bio-Rad) is a liquid culture medium designed to accelerate the growth of mycobacteria. The aim of this study is to prove the role of BioFM liquid medium in the isolation of mycobacteria from respiratory and nonrespiratory clinical samples of patients treated in Saint László Hospital for Infectious Diseases. The chromogenic BioFM liquid medium was evaluated and compared both with smear examination and with the conventional Löwenstein–Jensen (LJ) solid medium from all of the samples and with the PCR-based system for the detection of *Mycobacterium tuberculosis* from all respiratory samples.
Methods: A total of 50 routine clinical specimens with the preliminary suspect of mycobacterial infection were tested for the presence of mycobacteria. Specimens included 28 respiratory samples (sputum, BAL, pleural, and tracheal fluid) and 22 nonrespiratory (CSF, pus, lymph node, gastric juice, urine, bone marrow, tissues) samples. All specimens (except sterile samples) were decontaminated by the NALC-NaOH method, examined by microscopy stained by Ziehl–Neelsen and cultured on BioFM and LJ medium. PCR testing to detect *M. tuberculosis* was performed from all respiratory samples.
Results: A total of 12 mycobacterial strains were isolated from 50 clinical samples: 8 *M. tuberculosis* complex (including two multidrug resistant *M. tuberculosis* (MDR-TB)) and four *M. avium* complex (MAC) strains. In the 12 positive cases, four were smear positive, nine were positive on LJ medium and all of the 12 mycobacterial strains were positive in BioFM medium. The appearance of growth of *Mycobacterium* species in BioFM medium was 1–6 weeks earlier than on LJ medium. The isolation of the two MDR-TB strains was 2 weeks faster in BioFM medium than on LJ medium. Three strains belonging to MAC were not isolated on LJ medium (two smear-negative cerebrospinal fluid and one smear-positive pus samples) but cultured in BioFM medium. The identification and susceptibility testing of the strains were performed directly from BioFM medium. In all three cases where PCR testing was positive, the *M. tuberculosis* strain was cultured in BioFM medium as well.
Conclusion: In our study, the BioFM liquid medium proved to be an effective method to detect *Mycobacterium* infection. There was a significant difference in the time required for the isolation of mycobacteria. The combinatination of BioFM medium with other solid medium is an excellent culture method for the detection of mycobacteria.

P645 Comparison of Dio-TK and Löwenstein–Jensen media for primary culture of mycobacteria: a preliminary study for a new medium

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Objective: To evaluate the Dio-TK Medium for primary culture of mycobacteria in comparison with the Löwenstein–Jensen (L-J) medium.
Methods: A total of 456 samples (348 sputum, 46 bronchial aspiration, 31 pleural fluid, 12 biopsies, 10 bronchoalveolar lavage, five gastric lavage, three urine, and one intrathoracic fluid) recovered from 327 patients were studied. After standart decontamination and concentration procedure, each sample was then cultivated in Dio-TK Medium, Dio-TK Selective, Dio-TK Medium with *para*-nitrobenzoic acid and L-J medium in 37°C. Acid-fast staining was employed for smear preparation. Time and presence of growth on Dio-TK Medium was monitored graphically by Incubascan software. Growth on Dio-TK Medium was evaluated with the color change from red to yellow due to the indicator in the medium and presence of acid-fast bacteria on the medium by staining. Occurrence of green color on the medium indicated contamination. L-J cultures were checked visually twice a week and time of growth was recorded.
Results: Among the specimens, 298 (65.4%) were smear-positive and 158 (34.6%) were smear-negative. Mycobacteria grew in 299 (65.6%) and 279 (61.2%) of the specimens on Dio-TK and L-J media, respectively. Contamination ratios for Dio-TK and L-J were 3 and 4.4%, respectively. In smear-positive samples, 8 (2.7%) samples were culture-positive on L-J and culture-negative on Dio-TK, whereas 10 (3.4%) were culture-positive on Dio-TK and culture-negative on L-J. In smear-negative samples, one (0.6%) sample was culture-positive on L-J and culture-negative on Dio-TK; whereas two (1.3%) were culture-positive on Dio-TK and culture-negative on L-J and 18 (11.4%) samples were culture-positive on both Dio-TK and L-J media. Two atypical mycobacteria were differentiated in sputum specimens as *Mycobacterium avium* complex and MOTT. Mean time of growth on Dio-TK and L-J were 12.8 (min. 3, max. 30) and 25 (min. 20, max. 30) days, respectively.
Conclusion: Dio-TK is a new very useful and practical medium for rapid primary culture of mycobacteria. Further studies may be necessary for its standardization in the automated system.

P646 Investigation of the level of IgG, IgM and IgA antibodies in tuberculosis patients referred to TB reference center, PHLS, Ahwaz, Iran

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The detection of tuberculosis, especially in its early stages is difficult, the culture and biochemical tests despite being specific, are time consuming and laborious and the clinical features of the disease are not specific. So to eradicate the disease, it is important to improve diagnostic techniques so that the active disease can be treated at early stage. Since the level of antibodies is increased during the active phase of the disease, serologic techniques such as ELISA are of value in early diagnosis because these are among the rapid, reliable and less costly diagnostic methods for the detection of pulmonary tuberculosis. In present research, a total of 90 sera were enrolled in the study. This group consisted of 45 sera belonging to patients with clinical and radiologic signs and microscopic examination of sputum suggesting active tuberculosis, who referred to TB reference center, PHLS, Ahwaz and 45 sera from healthy subjects. Sera were obtained from the patients before receiving antituberculosis chemotherapy. ELISA test was used to determine the IgG, IgM and IgA antibodies activity against the A60 specific antigen of mycobacteria. The results revealed that in general antibodies especially IgG and IgA were much higher in tuberculosis patients compared with healthy subjects. There was no relevance between antibody titer and gender or previous BCG vaccination, but the relevance with age and the degree of smear positivity were significant. The titer of IgG and IgA was higher in patients under 50 years old and patients with 3+ sputum smear had significant increased IgG titer. Besides, the antibody titer was significant in culture-positive patients compared with those with a negative culture result. Present study showed that the ELISA using A60 antigen can greatly facilitate the diagnosis of tuberculosis especially in TB endemic area where a rapid, inexpensive, and reliable technique is most needed.

P647 Development of serologic test of *Mycobacterium tuberculosis* in humans

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Introduction: Serologic tests for the diagnosis of infectious diseases are very useful because they are technically straightforward and do not need isolation and culture of the pathogen. *Mycobacterium tuberculosis* causing approximately 1.5 million human deaths per year worldwide. Diagnostic tests which let us discern among individuals with active tuberculosis, infected, asymptomatic and noninfected individuals are needed. *M. tuberculosis* antigens induces a great and heterogeneous variety of antibodies. The aim of this work is to analysis and compare the antibody production against different mycobacterial antigens in individuals with the mentioned characteristics by the multiple antigen fix immunoassay (MAFI) test.
Methods: Employed antigens rPT-16, 38 kDa, 45/47 kDa, thioredoxin, ESAT-6 proteins; AN-5 and RV-11 culture filtrates from *M. bovis* and *M. tuberculosis*, respectively; and purified protein derivative (PPD) from

M. tuberculosis, *M. bovis* and *M. avium*. Ninety-nine serum samples were collected from 22 healthy individuals, 42 individuals with different types of tuberculosis, 30 tuberculosis suspicious individuals and five with other nonrelated diseases. The antigens were immunoblotted on nitrocellulose membranes and incubated with each serum sample. The membranes were then rinsed and incubated with antihuman IgG peroxidase. The peroxidase activity was detected with 4-chloro-1-naphtol and H2O2.
Results: 54.8% of the patients with tuberculosis recognized at least one of the analyzed antigens. From these, 100% recognized RV-11, 91.3% PPD tuberculosis, 86.9% AN-5 and PPD bovis, and 56.5% rPT-16. In the group of suspected infection individuals, 23.3% recognized at least one mycobacterial antigen of which 100% recognized RV-11, 85.7% AN-5 and PPD tuberculosis, 71.42% PPD bovis, and 57.14% rPT-16. Only one serum from the healthy individuals recognized RV-11 and PPD tuberculosis. With the exception of one sample from the patients with tuberculosis, none of the sera recognized PPD avium. The group of unrelated diseases did not recognize any of the antigens used.
Conclusions: The RV-11 culture filtrate, PPD tuberculosis and rPT-16 recombinant protein are promising antigens for the tuberculosis diagnosis using the MAFI technique. Recognition of the culture filtrate AN-5 and PPD bovis might reflect previous vaccination of the individuals.

P648 Serologic test for the diagnosis of tuberculosis in bovine cattle

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Introduction: Bovine tuberculosis, which is caused by *Mycobacterium bovis*, has a considerable negative economic impact for cattle farming and constitutes also a major risk factor in human public health. It is important to develop simple, fast, and inexpensive diagnostic tests, which do not need the isolation and culture of the pathogen and specialized equipment. Serologic techniques have the potential to fulfil these criteria. *M. bovis* may induce a highly diverse antibody response. But to date, there is no serologic test available, capable to distinguish healthy bovines from those having the disease; so the aim of this investigation is to analyze and compare the antibody production against different mycobacterial antigens in bovine cattle using the Multiple Antigen Fix Immunoassay (MAFI) technique.
Methods: Ninety-five sera samples were collected from Holstein Friesian bovine cattle: 22 were PPD positives (PPD+), 37 were PPD negatives (PPD–) and for 36 with bordeline results in the tuberculine test (PPD+/–). The antigens tested were 16 kDa, 38 kDa, 45/47 kDa, Thioredoxin and ESAT-6 proteins, H37Rv and AN-5 culture filtrates, and purified protein derivatives from *M. tuberculosis* (PPDt), *M. bovis* (PPDb) and *M. avium* (PPDa). The antigens were immunoblotted on nylon membranes and incubated with each serum sample. The membranes were then rinsed and incubated with anti-bovine IgG peroxidase. The peroxidase activity was detected with 4-chloro-1-naphtol and 0.01% H_2O_2 in PBS.
Results: 72.7% of PPD+, 13.8% of PPD+/– and 35.14% PPD– animals recognized at least one antigen. The PPD+ animals differentially recognized the antigens as follows: rPT16 (22.7%), RV-11 (72.7%), AN-5 (45.5%), PPDt (59%), PPDb (22.7%) and PPDa (13.6%). The corresponding values for the PPD – animal group were: rPT16 and 45/47 (5.4%), p-38 (2.7%), RV-11 (21.6%), AN-5 (13.5%), PPDt (21.6%), and PPDb (10.8%). The PPD+/– animals only recognized RV-11, PPDtub, AN-5 and PPDb in an interval of 13.8 to 2.8%.
Conclusion: The recognition of the antigens is heterogeneus for each animal gruop. However, the percentage values of recognition were strickingly higher for the PPD+ animal group than in the other two groups. The RV-11 and AN-5 culture filtrate, PPDtub and rPT16 recombinant protein are promising antigens for bovine tuberculosis diagnosis by the MAFI technique.

Non-molecular diagnostics: general

P649 Constructing and testing a novel system for the rapid identification of bacteria using MALDI-TOF-Mass Spectrometry

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Objectives: To assemble a mass spectral database of the ionizable components of bacterial cell surfaces generated by Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) and assess its potential for rapid identification of unknown isolates.
Methods: Strains tested were propagated and analyzed using optimized protocols. Data collection was carried out on the Micromass M@LDI mass spectrometer and data processed using the MicrobeLynx™ and MassLynx™ software (Micromass UK Ltd, Manchester). For the database, characterized strains from the National Collection of Type Cultures (NCTC), UK, representing clinical, environmental and food borne pathogens were used. Entries were quality controlled and validated by comparison of like spectra between Manchester Metropolitan University, Micromass UK Ltd and the Molecular Identification Services Unit, NCTC. Field isolates sent into the PHLS for identification were analyzed using the above system. The software was used to locate the closest matching spectra within the database and provided a probability score. A comparison with the identification based upon cellular fatty acid profiles and 16S rDNA sequence analysis was used to assess the accuracy of the MALDI identification.
Results: The MALDI-TOF-MS database (ca.1500 species) was essential for the identification of the test isolates and was achieved in a fraction of the time taken using other methods. Of 149 isolates, ~90% were identified correctly at genus level and 74% at species level including organisms that are typically difficult to identify. For example, nonfermentative, Gram-negative species which are often difficult to identify by most methods were readily characterized using MALDI-TOF-MS.
Conclusions: Identification using MALDI-TOF-MS correlated well with other techniques and represents a rapid and novel method for the characterization of microorganisms. Furthermore, sample preparation is minimal, only a small amount of bacterial growth is required and consumable costs are negligible. The technique should find wide application in clinical and reference laboratories.

P650 Evaluation of the VITEK 2 system for routine identification of clinical isolates in a Portuguese clinical microbiology laboratory

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Objectives: This study was performed to evaluate the quality of the performance of the VITEK 2 cards used for the identification of Gram-negative rods (GNR), Gram-positive cocci (GPC) and yeast isolates in the routine context of our microbiology laboratory.
Methods: A total of 250 GNR, 137 GPC and 13 yeast, composing a sample of species that are commonly isolated in our laboratory, were tested using the VITEK 2 ID-GNB, ID-GPC and ID-YST cards, according to the manufacturer's recommendations in comparison with the API strips (API 20 E, API 20 NE, ID 32 STAPH, API 20 Strep, ID 32 C) used as the reference method. Quality control strains were tested with each study batch. The identification obtained with the VITEK 2 system, were divided into four categories: correct identification (unambiguous correct identification at the species level); low level of discrimination (either identification at the genus level or low level of discrimination between several species, including the correct species); no identification (a doubtful, unacceptable, or unreliable identification); and misidentification the species identified with the VITEK 2 system was different from that identified by the reference method). Discrepant results were repeated in duplicate both on VITEK 2 and the reference method, strains with remaining discrepant results were sent to bioMérieux laboratory for retesting.
Results: Overall 95.8% of all strains were correctly identified at the species level (98.9% Enterobacteriaceae, 89.3% nonfermenters, 98.4% Staphylococci, 92.4% Streptococci, 100% Yeast). One strain of Enterobacteriaceae was not

identified (0.25%). Misidentifications were observed for 13 strains (3.2%), seven of these (54%) strains were nonfermenters spp., two *Citrobacter* spp., two *E. faecium* and two *Streptococcus alfa hemolyticus*. Twenty-three (5.7%) strains were identified to the species level after additional simple tests (indol reaction, motility and pigmentation). For 10 strains (2.5%) these additional tests did not give an identification at the species level, nevertheless the correct species identification was given among the organisms listed. Four strains were excluded from the study because no reference identification was obtained.
Conclusion: The VITEK 2 System demonstrates an excellent capability for providing a rapid and reliable means for the routine identification of clinical bacteria.

P651 Comparison of two methods for biochemical speciation of lactobacilli including probiotic strains

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Objective: Lactobacilli are important as probiotics; however, a simple standardized method for their speciation does not exist. Taxonomy of the genus is confused with many commercial strains assigned species names that have not been properly validated. 16S rRNA sequence analysis can be applied but does not on its own given information about probiotic attributes. Our aim was to determine a simple, reliable method for biochemical speciation and preliminary characterization of strains.
Methods: Forty-two isolates from probiotic and dairy products were used. Survey of the literature was used to compile a set of characters which include growth at different temperatures, tolerance to acid and bile, production of NH_3 from arginine, aesculin hydrolysis and the fermentation profile of 22 sugars to allow speciation according to accepted taxonomy. Two test formats were compared: (1) solid media inoculated using a multipoint inoculator (2) broth media in 96-well plates. With each, bromocresol purple (BP, read by eye) and chlorophenol red (CR, read by eye or at 450 nm with a plate reader) were evaluated as pH indicators. Test results were read at 2–14 days as appropriate. Cultures were incubated anerobically, and tested in duplicate.
Results: The most discriminatory tests for speciation were growth at 15 and 45 °C and sugar fermentation tests. Both methods were amenable to high throughput and identified *Lactobacilus acidophilus*, *L. casei casei*, *L. casei rhamnosus* in agreement with the probiotic products labeling. With method (1) there was interference between strains, where some reacted more quickly than others and the media in the plate was acidified. Method: (2) needed less anaerobic incubator space and substrates, and there was no interference between tests read at different times. With method (1) CR results were unreadable by eye as the color change was too subtle, duplicates with BP matched in 90% tests. Test reproducibility using method (2) was 95% with CR but 98.5% with BP.
Conclusion: The 96-well plate method with bromocresol purple indicator allows simple, reproducible speciation of lactobacilli and quantification of useful probiotic characteristics such as acid and bile tolerance.

P652 Comparison of methodologies for anaerobic organisms described in NCCLS document M40-P Quality Control of Microbiological Transport Devices

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Introduction: Prior to production of NCCLS document M40-P, Quality Control of Microbiological Transport Systems; Proposed Standard, there had not been a recognized procedure for determining the effectiveness of transport devices. Here we describe our experience using the new standard to evaluate six commercial swab transport systems.
Methods: ATCC strains of *Bacteroides fragilis*, *Propionibacterium acnes*, *Peptostreptococcus anaerobius*, *Prevotella melaninogenica*, and *Fusobacterium nucleatum* were inoculated to two different swab transport devices (Amies without charcoal and liquid Stuart's), from each of three manufacturers, Copan Diagnostics, Starplex Scientific Inc., and Medical Wire & Equipment Co. Swabs were held at ambient (20–25°C) and refrigeration (4–8 °C) temperatures for 0, 24, or 48 h, and then plated on Columbia agar containing 5% sheep blood. Viable bacteria were counted following incubation. Growth recovery was determined for both a roll-plate and swab elution technique. Base-line counts were obtained from media inoculated at zero time. Performance characteristics were compared with acceptance criteria described in M40-P.
Results: Trends in growth recovery were similar using either the roll-plate or swab elution techniques, irrespective of the organism, holding time or temperature. The Copan and Starplex transport devices met acceptance criteria for both roll-plate and swab elution techniques with *B. fragilis*, *P. acnes*, and *P. anaerobius*. The Medical Wire transport devices met acceptance criteria for *B. fragilis* and *P. anaerobius* using both techniques; however, failed to meet acceptance criteria for *P. acnes* for the roll-plate technique at ambient temperature. None of the transport devices tested met acceptance criteria for *F. nucleatum* and *P. melaninogenica* by either the roll-plate or swab elution techniques.
Conclusion: We found the new proposed NCCLS standards provide an excellent means of evaluating swab transport systems. The roll-plate method described in the document is well suited for most laboratories, whereas, we found the swab elution technique to be costly and labor intensive without yielding additional information.

P653 Comparison of two culture media to detect group B streptococcal-colonized pregnant women

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Objective: Comparison of direct plating on Granada agar plate and Instant Granada Medium agar tubes for detection of GBS colonization in pregnant women.
Methods: 517 vaginal and rectal exudates from pregnant women between 35 and 37 weeks of gestation were included in this study. In 320 patients, four separate swabs were collected in each woman, and in the other 197, two separate swabs were also collected. Samples were inoculated on a selective differential agar plate (Granada) and also were immersed in instant Granada medium (after rehydratation). The plates were incubated at 37 °C anerobical atmosphere for 24–48 h, and the tubes were incubated 24–48 h at 37 °C aerobically. In both media, the presence of intense-orange pigmented colonies is a characteristic of GBS.
Results: In the first group, 320 women studied with vaginal and rectum exudates separate by, 69 (21.5%) were positive in direct plating and 46 (14.4%) were positive in instant Granada medium. Three patients were positive in instant Granada medium and negative in agar plates and 26 women were negative in instant Granada medium and positive in agar plates. In the second group (197 women), 51 (25.8%) were positive in direct plating and 33 women (16.7%) were positive in instant Granada medium. In this group, all those positive in instant Granada medium were also positive in direct plating.
Conclusions:
1. When vaginal and rectal specimens were collected separate by 100% of GBS isolates detected were recovered in Granada plates and 66.6% in instant Granada medium.
2. When vaginal-rectal were collected together, only 64.7% of the positives women were recovered in instant Granada medium.
3. 41 of those women positive in both media (34.35%) will be lost if only instant Granada medium is used.
4. Granada agar plate is better for GBS detection in pregnant women than instant Granada medium.

P654 Occurrence of invasive GBS infections with serotype VIII in Denmark, 2001–2002

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Objectives: Group B streptococcus (GBS) (*Streptococcus agalactiae*) is a frequent cause of mortality and morbidity in neonates. The GBS serotypes Ia, Ib, II, III and V are the most prevalent among patients with invasive GBS disease in Europe and USA, while serotype VIII predominates among GBS isolated in Japan. Outside Japan, only a few isolates with serotype VIII have been described. We have identified four GBS serotype VIII isolates in Denmark in 2001–2002.
Methods: The Streptococcus Unit, Statens Serum Institute, Denmark, receives the majority of Danish invasive GBS isolates. After determination of the group B antigen, the isolates were serotyped by the precipitation method described by Lancefield. The antigens were extracted using both 0.1 and 0.2 N HCl. The invasive GBS isolates were from blood of patients admitted to hospitals from January 2001 to December 2002. In addition to the four clinical strains, two reference serotype VIII GBS strains (JM9 Prague no. 130013 and JM9 Prague no. 1330669) were analyzed by Pulsed Field Gel

Electrophoresis (PFGE). Antibiotic susceptibility testing of the clinical and reference strains was performed on blood agar by disc diffusion.
Results: The mean age of the four patients (two males and two females (not pregnant)) was 62.3 years and none of them had been outside Denmark in the last 3 weeks before admission to hospital. The clinical strains were identified in blood. One of the clinical strains shared an identical PFGE pattern with the two reference strains, and the clinical strains were assigned into two closely related DNA profiles. In addition, the susceptibility pattern showed minimal differences between the four clinical isolates.
Conclusion: We conclude that the four clinical strains were very closely related to each other and to the two reference strains although not completely identical. We believe that serotyping with all the nine type antisera (Ia – VIII) by use of different extracts (0.1 and 0.2 N HCl) can reduce the proportion of non-typable strains and result in a more complete and correct description of the serotype distribution in GBS surveillance programs.

P655 Use of synthetic antigens *Borrelia burgdorferi* sensu lato in immunoenzyme assay

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Objectives: Ixodes tick-born borreliosis (ITB) is a widespread disease in the Russian Federation. A multiplicity of clinical manifestations of the infection necessitates implementation of new reliable and inexpensive diagnostic laboratory tools into practice; improvement of serodiagnositics seems to be the most perspective trend. One of possible options could be a use of immunoenzyme assay (IEA) based on the artificial analogs of the natural borreliosis protein antigens.
Methods: We have studied six synthetic peptides imitating potential antigenic determinants of the 41 kDa, regions P35 and P39, borreliosis lipoproteins (two peptides from each region). Seventy-eight blood sera from patients with different clinical ITB manifestations and 34 blood sera from healthy donors were tested by IEA to study specific antibody/antigen reactions.
Results: Best results of immunochemical reactivity were obtained for the peptide 69–90 from the P35 region and peptide 119–339 from the P39 region (amino acid numbering according to the sequence of the natural antigen).
Conclusions: The results obtained shows a possibility to use synthetic peptides in immunoenzyme diagnosis of ITB. Additional investigation should be carried out to exclude possible nonspecific reactions while using borreliosis antigens for testing material from syphilis and other spirochetoses patients.

P656 Possibilities of immunoenzyme assay for diagnostics of early neurosyphilis

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Objectives: A total of 23 syphilis patients in different clinical periods (mean duration of disease 2 years) were examined. On the basis of clinical and laboratory data five of them were diagnosed to have early neurosyphilis. The specific lesion of the central neural system was ruled out for 18 patients.
Methods: For each patient a method of immunoenzyme assay (IEA) was used to detect antibodies to major *Treponema lipoproteins* with molecular weight of 15, 17, 41, and 47 kDa in blood sera and cerebrospinal fluid.
Results: In the majority of patients the antibodies to all four *Treponema* antigens were detected in blood sera. IgG1 and IgG3 subclasses were prevailing in the pool of antitreponemous antibodies. Comparative analysis of the level of antibodies of these subclasses did not reveal statistically significant difference between patient groups ($P > 0.05$). In cerebrospinal fluid the antibodies to the *T. lipoproteins* were found only in samples from the patients with confirmed diagnosis of early neurosyphilis. At the same time, all the samples were found to be positive in IEA for IgG1 to the 17 kDa antigen ($P < 0.05$). For all the patients with excluded syphilitic lesion of the central neural system, antibodies to the 15, 17, 41, and 47 kDa antigens were not detected in cerebrospinal fluid.
Conclusions: We thus propose a method of serodiagnostics of early neurosyphilis. Detection of antibodies to two or more *Treponema* antigens in the blood serum of the syphilis patient and detection of antibodies to 17 kDa antigen in cerebrospinal fluid only, reflect early syphilitic lesion of the central neural system.

P657 Evaluation of a new cellulose sponge for microbiological sampling

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Objective: We have developed a new type of sampling/transport swab (the Cellswab, Cellomeda, Finland), which is made of a highly absorbent cellulose viscose sponge material.
Methods: The survival of 14 aerobic, and 10 anaerobic and microerophilic bacterial species in the Cellswab, two commercial swab transport systems (Copan, Italy, and Orion Diagnostica, Finland) and one dacron swab (TSC, UK) was evaluated. Bacteria were suspended in broth, into which the swabs were dipped. Aerobic bacteria were stored in dry tubes, the others in transport medium, at 4 °C and RT, for up to 14 days.
Results: The Cellswab absorbed 1.3 times more, and released 3.5 times more fluid upon plating than the other swabs. Swabs were plated at 0, 1, 2, 4, 7 and 14 days. For 10 strains the Cellswab yielded $\geq$10% of the original CFU for longer than all the other swabs. It never performed worse than the other swabs. The Cellswab showed no inhibitory properties.
Conclusions: The Cellswab was found to perform at least as well as ordinary swabs. It was better at storing fastidious strains, and at keeping bacteria viable for long storage times; it might well be a useful replacement or complement to ordinary swabs.

P658 Evaluation results of 10th external quality assessment of microbiology laboratories of Tehran 2002

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Objective: The aim of this study was to evaluate performance of microbiology laboratories in Tehran.
Methods: In this study we distributed 487 unknown samples each containing two strains of bacteria between microbiology laboratories located in Tehran. Of 487, laboratories we received answers from 437 (89.7%) laboratories. Five species of bacteria including (1) *Staphylococcus saprophyticus*, (2) *Citrobacte freundii*, (3) *Acinetobacter baumanii*, (4) *Entrococcus faecalis*, (5) *Enterobacter agglomerance* were selected and randomly two strains of them sent to each laboratory. We asked for identification all of strains and susceptibility testing for *S. saprophyticus* and *C. freundii*.
Results: Of 291 laboratories, 224 (77%) produced correct answer for *S. saprophyticus*. Of 146 laboratories, 102 (69.85) for *C. freundii*. Of 114 laboratories, 34 (30%) for *Acinetobater baumanii*. Of 146 laboratories, 37 (25.3%) for *E. faecalis* and of 177 laboratories, 63 (35.6%) for *E. agglomerance*. A score of 3 points were given for correct identification of each strain, while for incorrect identification score of 0 point were given. The incorrect answers for above mentioned bacteria were 7.9, 27.3, 43.8, 26 and 52.5%, respectively. The other laboratories obtained of 0.5–2.5 scores of points for partially correct answers. The most laboratories were able correct performing susceptibility testing. Eighty-nine percent of laboratories reported correct susceptibility testing answer for *S. saprophyticus* and 79 for *C. freundii*.
Conclusion: It is concluded that most microbiology laboratories were not able to identify correctly unknown strains of bacteria, it may be due to lack of some reagents, culture media and experienced personnel.

P659 Predictive value of the detection of leukocytes and erythrocytes in feces for the diagnosis of inflammatory bacterial diarrhea

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Objectives: To evaluate the usefulness of the detection of leukocytes and erythrocytes in fecal wet preparation, as predictive markers for the presence of enteropathogenic bacteria cultured in the stools of adult patients with diarrhea.
Material and methods: During a 3-year period (from October, 1999 to November, 2002) 1230 of unformed stool specimens (one specimen/patient) were examined: (a) for leukocytes and erythrocytes by direct microscopy of wet preparation and (b) for the enteric pathogens *Salmonella*, *Campylobacter*,

Shigella, *Yersinia enterocolitica* and *Aeromonas* by the standard laboratory methods.
Results: Enteropathogenic bacteria were isolated in 174 out of the 1230 (14.15%) stool specimens examined by culture. In 156 (89.65%) and 143 (82.18%) of the 174 culture-positive specimens leukocytes (WBCs) and combination of leukocytes and erythrocytes (WBCs + RBCs) were detected, respectively. In 248 (23.48%) and 170 (16.09%) of the 1056 culture-negative specimens WBCs and WBCs + RBCs were detected, respectively. Of the 248 culture-negative patients with WBCs or WBCs + RBCs (170 of them had both) in the stools, the 179 (72.17%) had inflammatory bowel diseases (χ^2 test, 0.72 mean, 0.2845 standard error, 95% confidence interval 0.6615–0.7766), 1 (0.40%) amebic dysentery and in the 68 (27.42%) patients the cause of inflammatory diarrhea was not identified (most of them were considered as having inflammatory bacterial enteritis based on clinical presentation). The sensitivity, specificity, positive predictive value, negative predictive and likelihood ratio of the presence of WBCs and WBCs + RBCs in the feces as predictors of a positive stool culture for enteric pathogens was estimated to 89.65, 76.51, 38.61, 97.82, 3.81% and 82.18, 83.90, 45.68, 96.61 5.10%, respectively.
Conclusions: The detection of leukocytes or leukocytes and erythrocytes in the stools is a simple, rapid and inexpensive method with high sensitivity and specificity for predicting the presence of enteropathogenic bacteria in feces of patients with diarrhea. Furthermore, the detection of WBCs or WBCs + RBCs in stools with culture negative for enteric pathogens, highly suggests inflammatory bowel disease.

P660 Utility of Binax Now(R)-rapid urinary test in a series of 67 consecutive adult patients with bacteremic pneumococcal pneumonia

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Objectives: Bacteremic pneumococcal pneumonia (BPP) occurs in about one-third of patients with community-acquired pneumonia (CAP) due to *Streptococcus pneumoniae* and mortality is twice that in patients without bacteremia. Some recent guidelines recommend ambulatory treatment of patients in groups I and II (PORT-score). The objective of this study is to analyze the sensitivity of Binax Now(R)-rapid urinary test in the diagnoses of BPP.
Methods: We prospectively collected clinical and microbiological data of patients with CAP admitted in a tertiary-level hospital from January 2000 to December 2002. Binax Now(R)-rapid urinary test in concentrated urine samples and blood cultures were routinely performed. Patients with HIV infection or with severe immunosuppression due to organ transplantation, neoplasic or hematological diseases were not included in our series.
Results: 697 patients with CAP were evaluated during the study period (188 cases (31%) were classified in Fine risk-class I–II). Pneumococcal pneumonia was diagnosed in 193 patients (27.7%); 48 (25.5%) Fine risk-class I–II. BPP was identified in 67 (34.5%) cases; 23 (34.3%) Fine risk-class I–II (representing 12% out of the 188 total number of cases in these Fine risk-classes). Binax Now(R)-rapid urinary test in concentrated urine was performed in 53 out of 67 patients with BPP, being positive in 52 (98%).
Conclusions: In our series, 12% of CAP patients classified in Fine risk-class I–II had a BPP. Binax Now(R)-rapid urinary test has a high sensitivity in patients with BPP. A negative result of the Binax Now(R)-test in concentrated urine samples of patients with CAP may permit to rule out bacteremic pneumococcal pneumonia. This information could be useful to improve the initial decision to treat cases in Fine risk-class I–II as outpatients with oral antibiotics.

P661 Direct susceptibility testing of urines at outpatient clinics using system U3

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Objective: One of the most common out patient ailments is urinary tract infection (UTI). Since urinary pathogens are no longer uniformly susceptible to standard empiric regimens, susceptibility test data would provide reliable individual treatment. Directed UTI therapy will improve patient care, reduce social and health-care costs and most importantly promote the rational use of antibiotics in the community. Direct susceptibility testing of urine specimens at out patient clinics will provide results within 24 h and facilitate the upfront prescription of appropriate antibiotics.
Methods: We compared direct testing of urines using system U3, a new susceptibility test approved by the FDA, in comparison to standardized urine isolate testing. System U3 was used with three different Dipslide brands: MB-Dip 2 (Madaus, Germany), DS 103 A (Oxoid, UK) and Uricult (Orion, Finland). Direct testing was done at three out patient clinics in the Stockholm area by nonlaboratory personnel and pure culture testing at AB BIODISK. Dipslides set up with system U3 were also sent to AB BIODISK for a second reading.
Results: 71 urine samples and corresponding isolates: *E. coli* (61), *Enterobacter* spp. (1), *Klebsiella* spp. (4), *E. faecalis* (1) and Gram-positive cocci (4) were tested with ampicillin (AM), cefalexin (CX), nitrofurantoin (NI), norfloxacin (NX) and trimethoprim/sulphamethoxazole (TS). Susceptibility category agreement for direct urine testing compared with isolate testing were 92–100% (Dipslide Madaus), 94–100% (Dipslide Oxoid) and 92–97% (Dipslide Orion). CX results on Cled agar on Dipslides gave lower agreement (50%) but results on the same medium agreed at 95% level. Reader variations between trained and non-trained personnel were low (81–100% agreement) except for TS (70%) due to difficulties in reading of trailing end-points at the so-called 80% inhibition by non-laboratory personnel.
Conclusion: System U3 was found to be simple to set up and interpret and could be reliably used by nontrained personnel at out patient clinics. Direct testing of urine samples with system U3 on dipslides were substantially equivalent to the standardized pure culture testing.

P662 Efficiency of Sysmex UF-50 Urine Flow Cytometer in detection of urinary tract infection

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Background: Diagnosis of urinary tract infection (UTI) has been based more on clinical findings than laboratory tests: positive leukocyte field in chemical test strip screening has low specificity and not all bacteria produce nitrite. Visual microscopy is rather laborious, needs special skills, and the result may depend on the analyst. The result of bacterial culture is not available in acute situations.
Objectives: We evaluated the efficiency of an automated urine flow cytometer, Sysmex UF-50 (Sysmex Corporation, Japan), in detecting UTI.
Methods: The analyzer identifies RBC, WBC, squamous epithelial cells, transitional epithelial and renal tubular cells, bacteria, hyaline and inclusional casts, yeast-like cells, crystals and spermatozoa using argon laser flow cytometry. Patient samples from wards and ambulatory clinics, positive for leukocytes (>20/μL, $n = 86$) in chemical test strip screening, were analyzed with UF-50, conventional bacterial culture acting as the reference.
Results: Bacteria were identified with a sensitivity of 88% and specificity of 72% compared with the reference method. Negative predictive value was 90% and positive predictive value 70%.
Conclusions: Automated urinalysis with the UF-50 urine flow cytometer offers considerable savings in time and labor, and provides results with 79% concordance with bacterial culture.

P663 Serodiagnosis of prosthetic joint infection by lipid S ELISA

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Objectives: *Staphylococcus aureus* and low virulent skin microorganisms including coagulase negative staphylococci and *Propionibacterium acnes* are major causes of prosthetic joint infection. Differentiation between these infections and aseptic loosening of prosthetic joints is important as the diagnosis influences the clinical management of the patient and revision procedure. However, false positive revision cultures arising from skin contamination make the microbiological diagnosis of prosthetic joint infection difficult. The objective of this current study was to investigate a serological approach to facilitate the diagnosis of prosthetic joint infection due to staphylococci by determining serum levels of IgG to lipid S, a recently described exocellular short chain form of lipoteichoic acid, in patients with prosthetic joint infection and controls.

Methods: Fourteen patients with suspected prosthetic joint infection due to staphylococci were included in the study. These patients yielded identical microorgansisms from two or more clinical sites associated with the prosthesis and had elevated levels of conventional markers of infection including C-reactive protein, erythrocyte sedimentation rate and white blood cell count. Furthermore, 23 patients undergoing elective arthroplasty and 16 patients with suspected aseptic loosening and negative microbiology were included as controls. Serum levels of antilipid S IgG were determined by ELISA.

Results: Ten (71%) out of 14 patients with suspected prosthetic joint infection had elevated serum levels of antilipid S IgG whilst 21 (91%) out of 23 control patients undergoing elective arthroplasty had negative titres ($P < 0.0001$). Sixteen (100%) out of 16 patients with suspected aseptic loosening had no detectable antilipid S IgG ($P < 0.0001$).
Conclusions: The lipid S ELISA may facilitate the diagnosis of prosthetic joint infection due to staphylococci and assist in differentiating between septic and aseptic joint loosening.

Helicobacter pylori

P664 Molecular analysis of *vacA* and *cagA* genes of *Helicobacter pylori* isolates from Turkish patients with peptic ulcer disease and non-ulcer dyspepsia

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Objective: In this study, we sought to determine the cagA status and the distribution of vacA genotypes of *Helicobacter pylori* (Hp) isolates from patients with peptic ulcer disease (PUD) and non-ulcer dyspepsia (NUD).
Methods: Gastric biopsy samples taken from patients, diagnosed as either Hp-related PUD ($n = 13$) or NUD ($n = 16$) by endoscopic and biochemical criteria, were sent to laboratory and kept at $-70\,^{\circ}C$ until use. Following the extraction of bacterial DNA with proteinase-K and phenol–chloroform method, PCR was utilized to amplify vacA s, m1, m2 and cagA regions using specific oligonucleotide primer sets. A 785-bp vacA m region was also amplified with VA6F/VA6R primer set and the resulting PCR product was directly sequenced using Sequitherm Excel II DNA Sequencing Kit. Nucleotide sequences obtained in this study and from GenBank database were aligned with Clustal X version 1.81 and a phylogenetic tree was constructed with neighbor-joining method after Kimura 2 parameter correction using MEGA version 2.1 software. Reliability of the phylogenetic tree was confirmed with bootstrap analysis (1000 sets).
Results: Distribution of the vacA s and m genotypes and the cagA status of the isolates are shown in Table 1. PUD was significantly correlated with vacA s1m1 genotype ($P = 0005$) and cagA positivity ($P = 0008$). All vacA s1m1 genotype isolates were cagA positive ($P = 0000$). Clustering patterns of the isolates in phylogenetic analysis were in accordance with the PCR-based genotyping results.

Table 1 Distribution of vacA s and m genotypes and cagA status of Hp strains isolated from patients with PUD and NUD.

	PUD		NUD		
	cagA(+)	cagA(−)	cagA(+)	cagA(−)	Total
s1m1	11	–	5	–	16
s1m2	1	–	–	3	4
s2m1	–	–	–	–	–
s2m2	–	1	1	7	9
Total	12	1	6	10	29

Conclusions: Our findings suggest that cagA positivite-vacA s1m1 genotype Hp strains are significantly related to PUD, as described for patients from Europe. However, as some of the patients with NUD also harbor these strains, there may be other host or bacteria-related factors operative in the pathogenesis of Hp infections. Our data also suggest that PCR-based genotyping is an accurate, rapid and inexpensive alternative to sequence-based methods and suitable for large epidemiological studies.

P665 Differences of vacA allelic types of *Helicobacter pylori* strains: the tales of the two cities

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Objectives: In some countries, a particular geographic pattern of different *Helicobacter pylori* genotypes were seen. The aim of this study was to determine allelic differences between *H. pylori* strains isolated from two cities, Kocaeli (Turkey) and Hamburg (Germany).
Methods: Each gastric biopsy was obtained from a different patient who complaints of dispeptic symptoms. Strains were cultured on Mueller Hinton agar supplemented with 10% sheep blood and identified by urease, catalase and oxidase tests. Phenol–chloroform method was used for DNA isolation. The vacA alleles were identified by a PCR method.
Results: A total of 72 *H. pylori* isolates 35 strains from Germany and 37 strains from Turkey were used in this study. CagA genotypes were found 71.4%, 75.7% in Hamburg and Kocaeli, respectively. VacA alleles s1a-m1, s1a-m1a, s1a-m2, s1b-m2 and s2-m2 were observed 8.4, 38.4, 25.7, 5.6 and 16.8% in Hamburg strains, respectively. VacA alleles s1a-m1, s1a-m1a, s1a-m2, s2-m2 were found 8.1, 10.8, 59.5 and 21.6% in Kocaeli, respectively. It was no found s1b-m2 allele in Turkey isolates.
Conclusion: In this study, cagA status was not found significantly different between the strains isolated from Hamburg and Kocaeli. As for vacA gene, s1a-m1a allele was the most frequent in Hamburg strains, whereas s1a-m2 allele was in Kocaeli strains.

P666 Analysis of *Helicobacter pylori* 3′ area of CagA gene by PCR-RFLP

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Helicobacter pylori, (Hp) causes active chronic gastritis in the infected population, 10% of which develop gastric and duodenal ulcers. In addition, Hp is considered as the risk factor for gastric carcinomas and mucosa associated lymphoid tissue (MALT) lymphomas. The most studied and presented virulence factors for this pathogen are the cytotoxin (vacA) and its associated gene product (cagA). The majority of strains isolated from high risk patients (i.e. peptic ulcer or gastric cancer patients) were found to be cagA-positive. Thus this gene has been identified as a pathogenic factor for Hp. In this study Iranian *H. pylori* strains were isolated from gastric biopsies by bacterial culture. Following identity confirmation, genomic DNA was extracted and conserved genes such as the Hp ureC gene were amplified by PCR. Furthermore, two different segments of the cagA gene in the 3′ area were PCR amplified and categorized by restriction fragment length polymorphism, RFLP. Due to the high frequency of Hp infection in developing countries, previous studies have shown no association between cagA-positivity and gastrointestinal disorders. Thus, in order to identify relevant virulence markers for the Iranian population, the heterogeneity in the 3′ region of the cagA gene was studied. Accordingly, PCR-RFLP was performed and eight categories were identified. Non-parametric statistical analysis (Chi-square) determined the association between each of the identified RFLP profiles and the associated disease. This data demonstrates that only the C profile has a weak association ($P = 0.33$). The rest had no significant correlation. Therefore, further research is required to identify other CagPAI markers.

P667 Real-time PCR for detection of clarithromycin resistance in *Helicobacter pylori* clinical isolates and in biopsy samples

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Background: Mutations involved in clarithromycin (CLA) resistance in *Helicobacter pylori* are detected by different genotypic methods. The aim of

the study was to determine resistance to CLA in *H. pylori* clinical isolates by a novel Real-Time PCR performed in a LightCycler (LC) and to standardize PCR conditions to use it directly from gastric biopsies.
Material and methods: A total of 63 strains obtained from gastric biopsies were studied. In vitro susceptibility to CLA was determined by an agar dilution and resistance considered when MIC > 1 mg/L and intermediate when MIC = 0.5 mg/L. 42 resistant and 21 susceptible strains were included. DNA from the isolates was extracted by a previously described method (Ge & Taylor) and from biopsies by a commercially available kit (Gentra). Different conditions such as magnesium concentration, primer annealing temperature, probes and DNA amount and storage of DNA, were tested. A 425 bp fragment of the 23S rRNA was amplified in a LC (Roche Diagnostics) with two probes: fluorescein-labeled anchor probe and LC-640 fluorescent-labeled sensor probe (wild type sequence) separated by two nucleotides in the sequence (Designed by TibMolBiol, Germany). After standard amplification cycles a melting cycle was performed with continuous reading of LC-640 from 50 to 90 °C. A perfect match with a susceptible strain was indicated by a melting temperature of 64 °C. However, when a mismatched sequence was found, a melting peak at 56 or 60 °C was observed. Appropriate controls were run in each experiment. After DNA extraction the whole process was performed in 1 h.
Results: Among the overall 63 strains tested the distribution of MIC values were as follows: 21 with MIC < = 0.25 mg/L, 2 with MIC = 1 mg/L, 4 with MIC = 2 mg/L, 2 with MIC = 4 mg/L, 11 with MIC = 8 mg/L, 10 with MIC = 16 mg/L, 10 with MIC = 32 mg/L and 3 with MIC = 64 mg/L. The melting temperature of all susceptible strains were 64 °C. However, the resistant strains had a melting temperature of 56 or 60 °C (34/42). In five resistant strains heteroresistance was observed. Moreover, three strains with MIC = 1 or 2 mg/L showed he wild type pattern by using LC. Best results in biopsies were found when using 2 mM $MgCl_2$, 50 °C primer annealing, 4 µL DNA in a 20-µL final volume and 0.2 µM each probe.
Conclusions: A very good correlation among the agar dilution and detection of mutation involved in resistance to clarithromycin was found. The use of Real-Time PCR and the LightCycler is an accurate an rapid method to detect resistance.

P668 Rapid diagnosis of Clarithromycin-resistant *Helicobacter pylori* infection by using a double-probe fluorescent in situ hybridization assay

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Objectives: We have evaluated the use of a fluorescent *in situ* hybridization (FISH) assay directly from biopsies to determine simultaneously *Helicobacter pylori* infection and its susceptibility to clarithromycin treatment.
Methods: 26 gastric biopsies from ulcer-patients were examined. Samples were homogenized in 2 mL of selective broth, and a 500-mL aliquot was used for FISH detection. Samples were fixed with 4% paraformaldehyde solution for 4 h and then washed with 1% PBS buffer. HPY probe, a 16S rRNA targeted FITC-labeled oligonucleotide sequence was used for the detection of all *H. pylori* strains. CLA1-3, a set of three CY3-labelled probes, was used for the detection of 23S rRNA mutations associated with resistance to clarithromycin. Hybridization was performed at 46 °C for 2 h. Results were compared with direct PCR, culture of samples on Pylori Selective Agar and Clarythromicin sensitivity *E*-test of isolated *H. pylori* strains.
Results: FISH allowed the detection of *H. pylori* in 20 out of 26 samples analyzed. Seven of these positive biopsies showed to contain Clarithromycin-resistant *H. pylori* strains. PCR detection yielded positive results for 15 samples. Strains could only be isolated from 14 biopsies. Results from *E*-test showed good correlation with FISH-detected resistance to clarithromycin.
Conclusions: FISH method can be used as a rapid and accurate way of diagnosis of *H. pylori* infection, and simultaneous evaluation of the optimal treatment for its eradication.

P669 Resistance of *Helicobacter pylori* isolates to metronidazole, clarithromycin, tetracycline, amoxicillin and cefixime in patients after treatment failure

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Objectives: Antibiotic resistance of *Helicobacter pylori* isolates differs according to geographical area. The aim of our study was to determine the resistance of *H. pylori* isolates to five antimicrobial agents from patients after treatment failure.
Methods: Sixty-six isolates of *H. pylori* were isolated from biopsy specimens of 66 dyspeptic adults (37F/36M; median age 57 years) after treatment failure with 2–4 commonly used antibiotics for *H. pylori* eradication. Susceptibility for metronidazole (MET), clarithromycin (CLR), tetracycline (TC), amoxicillin (AM) and cefixime (CEF) was tested by *E*-test (AB Biodisc). Isolates were considered as resistant if MIC for MET ≥ 8 µg/mL, for TC ≥ 4 µg/mL for AM ≥ 1.5 µg/mL, for CLR and CEF ≥ 2 µg/mL.
Results: Resistance to MET and CLR was found in 53 and 51.5% of isolates, respectively. Dual resistance to both antibiotics was detected in 27.3% of isolates. High MIC value of ≥256 µg/mL for MET and CLR was observed in 37.8 and 24.2%, respectively, 97 and 91.4% of isolates resistant to CLR or MET were recovered from patients previously treated with CLR or MET, respectively. One isolate was found to be resistant to AM and TC and to all other antibiotics with MIC ≥ 256 µg/mL. This isolate was recovered from patient previously treated with four antibiotics: MET, CLR, TC and AM. Resistance to CEF was found in 4.5% of isolates.
Conclusion: High resistance to MET and CLR was found in patients previously treated with MET and CLR, respectively. CEF which is not currently used for treatment of *H. pylori* and TC are potential alternative agents for eradication after treatment failure.

P670 Antimicrobial potential of lactobacilli against reference and wild *Helicobacter pylori* strains

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Objectives: To screen for antagonistic activity of different lactobacilli against *Helicobacter pylori* wild and reference strains in vitro.
Methods: In total four lactobacilli strains from different origins were included: *Lactobacillus fermentum* IV from gastric mucosa, *Lactobacillus salivarius* 180-2-1 from the fecal sample of healthy young Estonian children, and two reference strains (*Lactobacillus rhamnosus* ATCC 53103 and *Lactobacillus paracasei* ssp. *paracasei* DSM 20020). Nine randomly selected *H. pylori* isolates from gastric samples and reference strain *H. pylori* NCTC 11637 were used. Antagonistic activity was assessed by a streak line procedure on Columbia Agar Base supplemented with 7% horse blood and 1% Vitox. A single line of lactobacilli suspension in 0.9% saline solution by McFarland 1.0 was seeded in the middle of the agar plate and incubated for 48 h in a 10% CO_2 environment at 37 °C. From the *H. pylori* culture, a suspension in Brucella broth of McFarland 3–4 was made and seeded in duplicate and perpendicular to the streak line of lactobacilli. Following incubation for 3 days at 37 °C in a microaerobic environment, the width of clear zone of inhibition (mm) extending from the culture line of lactobacilli was measured.
Results: Although the tested lactobacilli strains inhibited the *H. pylori* strains investigated, inhibition of the reference strain *H. pylori* (NCTC 11637) was more effective than for the wild types. The inhibitory activity of the lactobacilli was not solely related to metabolic type of the lactobacilli, suggesting a role of strain-specific properties of lactobacilli in the suppression of *H. pylori*.
Conclusions: The in vitro tests showed the contrast between the susceptibility pattern of reference strain and wild isolates of *H. pylori* to distinct lactobacilli with probiotic properties. While selection of an individual and suitable probiotic strain is complicated, the application of some combination of effective lactobacilli strains could be recommended (Table 1).

Table 1

	Inhibition zone in mm (median/ranges)			
	L. salivarius 180-2-1	*L. paracasei* ssp. *paracasei* DSM 20020	*L. rhamnosus* ATCC 53103	*L. fermentum* IV
NCTC 11637	19.0/15-23	18.0/15-20	19.0/18-22	17.5/16-18
HP2	21.0/20-22	13.0/12-15	18.5/17-20	8.5/8-10
HP1	12.0/12-14	6.0/5.7	7.0/6-7	0
HP6	12.0/11-13	9.0/6-10	11.0/10-12	0
HP7	7.0/6-10	6.0/5-7	0	0
HP8	6.0/5-7	7.0/6.8	0	0
HP9	13.5/13-15	7.5/6-8	8.0/8-10	0
HP3,4,5	0	0	0	0

P671 Dental plaque and *Helicobacter pylori* infection in Iran

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Objective: The precise mode of transmission and the natural reservior for *Helicobacter pylori* are unknown. The aim of this study was to investigate the prevalence and distribution of *H. pylori* in dental plaque and its correlation with *H. pylori* infection of the stomach.
Methods: Samples were obtained from supragingival and subgingival dental plaques and gastric mucosa (antrum and body) of 78 dyspeptic patients (age range 11–90 years, 45 men, 33 women) with endoscopically active chronic gastritis, peptic ulcer and gastroesophageal reflux (with normal gastric mucosa). *H. pylori* infection was evaluated with rapid urease test, histology and culture. For detection of *H. pylori* in dental plaque, rapid urease test, Gram staining, culture and histology were performed.
Results: *H. pylori* was detected in antral and body gastric mucosa specimens of 87% (68 of 78) by rapid urease test, 85% (66 of 78) by histology and 66% (52 of 78) by culture. All (100%) of 78 patients under study, were positive for *H. pylori* colonization in dental plaque by rapid urease test, Gram staining and histology. By culture 72% (56 of 78) of *H. pylori* infected patients were positive for colonization of the bacterium in dental plaque. *H. pylori* was detected in all three locations but with differences in prevalence: 89% in the molar region, 61% in the premolars and 42% in the incisors. Dental plaques of 55.1% of *H. pylori* infected patients, 5.1% of chronic gastritis who were not infected with *H. pylori* and 6.4% of patients with normal gastric mucosa were positive by rapid urease test within 10 min.
Conclusions: The results of this study show that *H. pylori* is present in dental plaque samples independent of the infection status of the stomach and may belong to the normal oral microflora.

Bloodstream infections in immunocompromised hosts

P672 Etiology of septic episodes in children with hematologic malignancies and solid tumors

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Objectives: Bacteremia is still a common and serious complication during the course of intensive chemotherapy for malignant diseases of hematologic origin and solid tumors. The aim of our study was to investigate the incidence and etiology of septic episodes during chemotherapy in the department of Pediatric Hematology – Oncology of our hospital.
Methods: We studied 111 children (43 girls and 68 boys, aged from 6 months to 14 years) suffering from hematologic malignancies and solid tumors, 63 and 48, respectively, during the August 2000 – July 2002 period. Among the hematologic malignancies 45 children (40.5%) had acute lymphoblastic leukemia, 8 (7.2%) acute myeloblastic leukemia, 9 (8.1%) lymphomas, 1 histiocytosis whereas 13 (11.7%) had different sarcomas, 12 (10.8%) neuroblastoma, 9 (8.1%) CNS tumors, 6 (5.4%) nephroblastoma and 8 (7.2%) other tumors. These patients developed 43 episodes of sepsis during the courses of intensive chemotherapy or relapse of their primary disease. Two hundred and forty-four blood cultures were obtained using pediatric Plus F vials from Becton-Dickinson and the Bactec 9240 blood culture monitoring system (B–D).
Results: Seventy-one blood cultures had proven positive (29%). Gram-positive bacteria accounted for 39.4% and Gram-negative ones for 60.6%. Fungi were not detected. Among Gram-positive bacteria, coagulase negative Staphylococci predominated (19/26, 8%) followed by *S. aureus* (6/8, 4%) and *Streptococci viridans* (3/4, 2%). *E. coli* predominated among Gram-negative bacteria (15/21, 1%) followed by *Klebsiellae pneumoniae* and oxytoca (13/18, 3%), *P. aeruginosa* (6/8, 4%), *Acinetobacter* spp. (4/5, 6%), *E. cloacae* (4/5, 6%) and *S. enteritidis* (1). The majority of septic episodes concerned children with hematologic malignancies (32 episodes against nine concerning solid tumors) $P = 0.007$. All septic episodes were treated successfully.
Conclusions: Septic episodes are more frequent among children subjected to intensive chemotherapy for hematologic malignancies than children for solid tumors. Gram-negative bacteria exceed Gram-positive ones, while the opposite was the case some years earlier.

P673 Infectious complications in patients with hematological malignancies consulted by the infectious diseases team: a retrospective cohort study

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Background: Although there has been significant advances in the management of infectious complications, they are still a major cause of morbidity and mortality in patients (pts) with hematological malignancies (HM).
Objectives: To identify the characteristics of pts with HM in the presence/suspicion of any accompanying infectious disease and the predictors of mortality in this group.
Methods: The hospital charts of patients with HM consulted by the Infectious Diseases (ID) team for signs/symptoms of any infection between January 1, 1997- December 31, 2001 were retrospectively evaluated. Data were collected on some demographic and disease characteristics and hospital outcome of the study participants. Uni- and bi-variate analyses were conducted for a total of 1132 episodes but multivariate analysis of predictors of mortality was limited only to the first episode of each participant.
Results: A total of 1132 consultations were done for 641 patients, 59.4% were men. The mean of age was 47.87 ± 1.36 years. The most common underlying diseases were non-Hodgkin's disease (30.9%) followed by acute myelogenous leukemia (26.2%). The underlying disease was in remission in 22.5% of all pts. Pneumonia was the most common clinically documented infection ($n = 173$). Bacteremia was detected in 122 episodes, 56.5% being due to Gram-negative microorganisms. Invasive fungal infection was detected in 51 attacks, 20 of which were pulmonary aspergillosis. A total of 163 patients died during the infectious episode.

In logistic regression analysis, the presence of pneumonia [OR = 7.56 (95% CI = 4.84–12.486)], fungal infection [OR = 4.12 (95% CI = 1.78–9.55)], relapse or recent diagnosis of underlying disease [OR = 2.82 (95% CI = 1.53–5.21)] and neutropenia, [OR = 2.70 (95% CI = 1.70–4308)], were identified as statistically significant predictors of mortality, controlling for age and gender.
Conclusions: Gram-negative and Gram-positive microorganisms are almost equally responsible for bacteremia. Patients with relapsed or newly diagnosed HM, who are neutropenic, have pneumonia or invasive fungal infection are more likely to have a poor prognosis. Therefore, we should act more aggressively while dealing with patients who have these risk factors.

P674 Outcome of catheter-related bloodstream infections in adult hematology patients

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Objective: To evaluate the impact of central venous catheter (CVC) removal on outcome of catheter-related bloodstream infection (CRBSI) in adult hematology patients.
Methods: Subjects were identified retrospectively according to diagnosis coding of 'sepsis' and subsequent inclusion was dependent upon the examination of medical records confirming both sepsis and a strong clinical suspicion that the source was the CVC. Demographic and bacteriological data as well as therapeutic measures and clinical outcomes were recorded.
Results: One hundred and three patient episodes were evaluated; the most frequent type of CVC was the Hickman catheter and the most frequently isolated pathogen was coagulase-negative staphylococci. Treatment failure, defined as recurrence of infection within 90 days or mortality attributed to sepsis within 30 days, occurred significantly more frequently in the group managed without catheter removal (52.5 vs. 4%, $P < 0.05\%$). Recrudescence within 90 days occurred in 36 (35%) episodes; this accounted for 46% of the 78 whose infections were managed without catheter removal; of these 36 episodes where recurrence was noted, 26 (72%) were associated with CoNS and 10 (28%) were associated with Gram-negative pathogens. There was no

significant difference between the two groups in respect of measured characteristics, other than CVC removal, considered as potential determinants of outcome such as gender, presence of neutropenia pathogen isolated and duration of antibiotic therapy.
Conclusion: More frequent CVC removal for CRBSI, in this population, is likely to be of benefit since not doing so is associated with treatment failure, morbidity and carries significant resource implications.

P675 An increase in methicillin-resistant *S. aureus* bacteremia in neutropenic patients

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Aim: The aim of this study was to analyze the spectrum of pathogens isolated from patients with neutropenic sepsis.
Methods: Blood cultures taken from neutropenic patients under the care of hematology and oncology services from 1st January 1999–30th September 2002 were analyzed.
Results: 3642 cultures were taken over the study period. Six hundred and sixty-eight were positive giving a positivity rate of 18.34%. The most common isolates were coagulase-negative staphylococci (42%), coliforms (18%), *S. aureus* (14%) and *Enterococcus* spp. (8%). There was no significant shift in the spectrum of pathogens with Gram-positive organisms predominating in each year. Fifty percent of *Candida* spp. isolated were identified as *C. albicans*. The spectrum of organisms observed between hematology and oncology patients was similar. With regards to the incidence of resistant organisms: 40% of *S. aureus* were methicillin resistant and 6% of enterococci were vancomycin resistant. Of the Enterobacteriaceae isolated, 2.6% were gentamicin resistant and 4.3% ciprofloxacin resistant. There was an increase in methicillin resistance in *S. aureus* isolates over the study period from 13% in 1999, 19% in 2000, 38% in 2001 and 75% in 2002, with the opposite trend observed in coagulase-negative staphylococci; 58% in 1999, 52% in 2000, 44% in 2001 and 40% in 2002.
Conclusion: Whilst recent studies have reported an increase in Gram-negative pathogens, Gram-positive cocci remain the most prevalent organisms isolated from blood cultures in our center. The incidence of multiresistant Gram-negative pathogens remains low. However there is a worrying increase in the incidence of methicillin resistance among *S. aureus* isolates, but all isolates to date remain sensitive to our current empiric antibiotic regimen.

P676 Micro-organisms isolated from neutropenic patients in a university hospital in Turkey with a special interest in non-fermenter Gram-negative bacilli

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Objectives: As early administration of appropriate antibiotics is crucial for successful management of infections in neutropenic patients, close monitorization of type and susceptibility of causative agents is mandatory both for the decision of empirical antibiotic therapy and evaluation of new antimicrobial drugs. This study is performed to show the microorganisms isolated in blood cultures of neutropenic patients with hematological malignancy in our University Hospital.
Methods: During the year 2001, we obtained 125 microorganisms from 121 febrile neutropenic episodes.
Results: Seventy-one (56.8%) of the isolates were Gram-negative bacteria (enterobacters 44, non-fermenters 27) and 43 (34.4%) were Gram-positives (cocci 40, coryneforms 3). The predominant bacteria were *E. coli*, *Klebsiella* spp. and coagulase negative staphylococci. The list of the isolates are shown in Table 1.

Table 1 The types of microorganisms from positive blood cultures

Bacteria	*n*	%		*n*	%		*n*	%
Gram positive	43	34.4	Gram negative	71	56.8	*Candida* spp.	10	8
Staphylococus spp.	27		Enterobacteriaceae	44	35.2	*C. albicans*	6	
S. aureus	12		*E. coli*	23		*C. tropicalis*	3	
CNS*- *S. epidermidis*	10		*Klebsiella* spp.	15		*C. glabrata*	1	
other CNS	5		*Enterobacter* spp.	4				
Enterococcus spp.	9		*Proteus mirabilis*	1		Others		
Streptococcus spp.	4		*Pantoea* spp.	1		*Haemophilus* spp.	1	0.8
Gr A beta hem. Strep.	1		Nonfermenters	27	21.6			
S. pneumoniae	1		*Acinetobacter baumannii*	7				
alpha hem. Strep	2		*Alcaligenes* spp.	1				
			Pseudomonas spp.	13				
Coryneform bacteria	3		*Stenotrophomonas maltophilia*	6				

CNS*: coagulase negative staphylococci.

Conclusion: Although a shift towards Gram-positive bacteria among isolates from neutropenic patients are observed in many countries, Gram-negative bacilli still seem to be the major pathogens in our hospital. When compared with a previously published data from the same hospital in 1998, the most considerable finding is the increase of non-fermenter Gram-negative bacilli (8.8 to 21.6%), with a marked increase in *Stenotrophomonas maltophilia* (0.8 to 5%) in three years time.

P677 *Stenotrophomonas maltophilia* bacteremia in cancer vs. noncancer patients. Comparison of risk factors, outcome and antimicrobial susceptibility

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Objectives: To compare two periods of survey 1992–95 and 1996–2001 for antimicrobial susceptibility and mortality of Gram-negative bacteremia.
Methods: A total of 61 episodes of *Stenotrophomonas maltophilia* bloodstream infections in 61 patients from 8 major teaching hospitals appeared between 1991 and 1995 and 1996–2001. This multicentric study included The National Cancer Institute, The National Institute of Heart Diseases and The Pediatric University Hospital in Bratislava and 5 other University Hospitals in Bratislava, Trnava, Nitra, Banska Bystrica, Kosice, Slovakia (5.5 milion inhabitants).The following clinical characteristics were recorded: sex, age, presence of underlying disease, previous colonization, source of infection, antimicrobial susceptibility, number of positive blood cultures, presence of vascular catheters, ventilatory support, dialysis, prior receipt of antibiotics (prophylaxis or therapy), previous surgery. Complications (endocarditis, abscess, pneumonia), oucome and therapy were also recorded. Strains from blood cultures were isolated through the semiautomated system Bactec or Bact-Alert and identified with Vitek Jr. system (Bio Mérieux).
Results: Sixty-one cases of *Stenotrophomonas maltophilia* bacteremia were analyzed. Thirty occurred in 1991–95 and 30 in 1996–2001, so there was no found of increasing occurence. Concerning risk factors there was no difference in major risk factors, cancer as underlying disease, whereas majority of cases in 1991–95 were from cancer patients in contrast to only 10% in 1996–2001. Relapse rate was significant higher in 1991–95 (10 vs. 0%) and attributable mortality, what was some what lower 3.3% in 1996–2001. Concerning antimicrobial susceptibility, there was lower resistance to colimycine and ceftazidime in 1996–2001, where susceptibility increased from 38.7% to 50% in colimycine and quinolones and 64.5% to 93.2% for ceftazidime ($P < 0.04$), respectively.
Conclusion: There are less attributable deaths and complications due to bacteremia caused by *S. maltophilia* and antimicrobial susceptibility improved within last 5 years when analasing two subgroups of patients with *S. maltophilia* bacteremias in 1996–2001 in comparison to 1992–95.

P678 *Stenotrophomonas maltophilia* infection in cancer patients – frequency, spectrum of infection and antimicrobial susceptibility

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Objectives: Frequent use of empiric broad-spectrum antibiotic therapy in cancer patients might result in the selection of some resistant organisms. Our objectives for this study were to ascertain the current frequency of infection, determine the spectrum of infection, and the antimicrobial susceptibility of *S. maltophilia* isolated from cancer patients.
Methods: (i) Review of institutional surveillance data (1986–2002) to determine frequency. (ii) Review of microbiological data to determine spectrum of infection. (iii) Microtiter, broth-dilution studies to determine in vitro susceptibilities to 22 antimicrobial agents.
Results: Surveillance data indicate that despite an overall decline in the frequency of Gram-negative infections in our cancer patients, there was an increasing frequency of *S. maltophilia* with these organisms causing 2% (22 of 851) of all Gram-negative infections in 1986; 3% (23 of 679) in 1993; 6% (45 of 758) in 1996; and 7% (61 of 903) in 2002. *S. maltophilia* rose from being the 9th most common Gram-negative isolate in 1986 to the 5th most common in 2002. The spectrum of infection included bacteremia (32%) colonization (27%), simple and complicated urinary tract infection (18%), pneumonia (12%), skin and soft tissue (4%), and other sites including disseminated

infection (7%). The most frequent site of colonization was from respiratory specimens. Antimicrobial susceptibility was variable with 76% of isolates being susceptible to clinafloxacin, 72% to TMP/SMX, 68% to cefepime, 66% each to gatifloxacin, moxifloxacin, and ticarcillin/clavulanate, and 64% each to ceftazidime and trovafloxacin. All other agents inhibited less than 50% of isolates. Eight percent of isolates were resistant to all agents tested.
Conclusions: The frequency of *S. maltophilia* infection in cancer patients has more than tripled in the past 16 years. The spectrum of infection ranges from disseminated disease to local colonization. Antimicrobial susceptibility is variable with no single agent inhibiting >76% of isolates. Some isolates are multidrug-resistant and might require high-dose, combination therapy.

P679 *Francisella philomiragia* bacteremia in a patient with chronic granulomatous disease

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Objective: *Francisella philomiragia* is a rare pathogen in humans. It has been found in patients with chronic granulomatous disease (CGD), near-drowning patients and patients with malignant bone marrow diseases. We describe a fatal case of bacteremia with this pathogen in a 24-year-old man with CGD. The patient, who was on holiday swimming and taking mudbaths in Turkey, developed high fever despite CGD-antimicrobial chemoprophylaxis with cotrimoxazole. One week after returning to Denmark, he was admitted to the ICU with clinical sepsis, pneumonia, pleural effusion and abdominal pain. Blood cultures were drawn and antimicrobial treatment was initiated with penicillin, ciprofloxacin and metronidazole. Penicillin was replaced by erytromycin on suspicion of Legionnaire's disease. The patient died from multiorgan failure and DIC on day 5. Four of five sets of blood cultures were positive with Gram stain only revealing an amorphous Gram-negative mass. Wet-mount microscopy showed spherical bodies with diameters of 3–5 μm, with only an occasional bacilli. Conventional identification was hampered by difficulty in determining microscopic morphology and weak and delayed phenotypic reactions. The diagnosis *F. philomiragia* was confirmed by 16S rDNA sequencing. Halophilicity was not demonstrated until the identification was established.
Conclusion: Familiarity with *F. philomiragia*, a very rare and difficult to identify pathogen, is important when dealing with CGD patients because of the organism's ability to cause serious disease and death. CGD patients should probably be cautioned about participation in salt water activities.

P680 Meropenem is effective and well tolerated in the treatment of febrile episodes in elderly or renally impaired neutropenic patients

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Objectives: Treatment of elderly patients, a significant and increasing proportion of the population, is often complicated by the presence of underlying illnesses, concomitant medication and decreased renal function. The dosage of predominantly renally excreted drugs needs to be carefully considered prior to the treatment of elderly patients. This study evaluated the clinical efficacy and tolerability of meropenem (MEM) in the treatment of febrile neutropenia in patients aged >64 years (or who were aged <65 years but renally insufficient [CrCl <51 mL/min]) (Group 1) compared with patients aged <65 years with normal renal function (Group 2).
Methods: An observational, prospective and comparative, open study was performed in 318 hospitalized, febrile neutropenic patients (aged >18 years) from 30 Spanish hospitals. MEM (1 g i.v.) was administered every 8 h for at least 7 days. The protocol allowed for dosage reductions in renally impaired patients (according to the manufacturer's guidelines). Clinical evaluations (EORTC criteria) including hematology, biochemistry and microbiology were performed on initiation, 72 h after, and at the end of treatment.
Results: Three hundred and eighteen patients were recruited to the study, 313 were evaluable for efficacy and 318 were evaluable for safety. Discounting two patients for whom there were no creatinine clearance values available, the mean age of Group 1 was 70.4 years (range 20–85, $n = 135$) and of Group 2 was 45.6 years (range 15–64, $n = 181$). The mean creatinine clearance at baseline was: Group 1, 58.7 mL/min; Group 2, 102.4 mL/min. 38.5% of the patients in Group 1 and 56.9% of the patients in Group 2 received antibiotic treatment prior to recruitment, and 54.5 and 70.1%, respectively, received concomitant antibiotic therapy (mainly glycopeptides and aminoglycosides) during the study. MEM was clinically and microbiologically effective in both groups. MEM was well tolerated in both groups and the incidence of nausea and vomiting was low. Importantly, in renally impaired or elderly patients no cases of seizures were reported. Efficacy outcomes, and incidence and types of adverse events are given in the Table 1.

Table 1

	Group 1 (>64 years or renally impaired)	Group 2 (<65 years with no renal impairment)
Clinical outcome at the end of the study (%)		
Success	64.9	60.6
Failure	26.1	32.6
Non-evaluable	9.0	6.9
Microbiological outcome at the end of the study (%)		
Eradication/presumed eradication	47.3	46.4
Persistence	1.5	6.0
Recurrence	1.2	1.2
Superinfection	0.8*	3.0*
Non-evaluable	50.4	43.5
Patients with adverse events (%)	2.2	3.3
Types of adverse events		
Renal insufficiency	2	
Diarrhea	1	
Liver disease	1	
Bronchospasm		1
Cutaneous rash		1
Nausea		1
Vomiting		1
Seizure		1
Intrahepatic choleostasis		1

*Within the expected range.

Conclusions: MEM was well tolerated and efficacious in the treatment of febrile neutropenia in the elderly and renally impaired as well as in patients aged <65 years without renal impairment.

Clinical infections and immunity

P681 Severe eosinophilia during the course of toxic shock syndrome

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Objectives: Staphylococcal toxic shock syndrome (STSS) is defined as the occurrence of fever, rash, hypotension, multiple organ dysfunction, and desquamation. It is caused by toxigenic strains of *Staphylococcus aureus*. The main hematologic change is usually thrombocytopenia and leukocytosis. Eosinophilia has been recently described first time by our unit in 50% of 20 cases with STSS. We described a patient with non-menstrual recurrent TSS going with severe eosinophilia.

Case report: A 16-year-old girl reported an episode of fever and rash beginning nearly one month ago. She had presented to a dermatologist 1 week later and treated with antibiotics for 10 days. She had responded partially to the treatment, but after discontinuation, the clinical features had appeared 1 week before admission. She had presumed psoriatic lesions on scalp and elbow for a few years and epilepsy for one year treated with carbamazepine. Temperature was 39.2°C, pulse 112 beats/min, and blood pressure 80/50 mmHg. A diffuse macular erythroderma and peeling on face, scalp, and palms and soles were noted. WBC was 34 300/mm^3 (eosinophils 62%), Hb 12.4 g/dL, platelets 205 000/mm^3, urea 10 mg/dL, cr 0.88 mg/dL, ALT 15 U/L, AST 17 U/L, CK 23 U/L, CRP 30.5 mg/L (0–5), and sedimentation rate 20 mm/h. CMV IgM, rubeola IgM, and Monotest remained (–). Samples from scalp cultured MRSA, swabs of throat, axilla, nose, and

umbilicus yielded MSSA. Carbamazepine was discontinued and valproate initiated. Teicoplanin was prescribed. IgE level was normal. No parasites were determined. Three days after the initiation of the antibiotics, her temperature returned to normal. The rash also resolved. A very prominent desquamation was noted on palms and soles on day 9. WBC count decreased to 15 000/mm^3 (eosinophils 32%). The dose of teicoplanin was decreased to 6 mg/kg/day. However 2 days after lowering the dose, another febrile episode with rash occurred. Repeated cultures yielded MSSA from the same sites. The treatment was replaced with amoxicillin/clavulanate and fusidic acid. She responded well in 2 days. After an afebrile period of 8 days, WBC count decreased to 7500/mm^3 (eosinophils 12%; 900/mm^3), and the treatment was discontinued. During a follow-up of 10 months, she has been doing well.
Conclusion: Our patient had recurrent non-menstrual STSS most probably facilitated by psoriatic lesions. Eosinophilia can be seen during the course of STSS in a considerable frequency and it can be severe.

P682 Antibodies to human heat shock protein 60 and to *Chlamydia pneumoniae* in abdominal aortic aneurysm patients

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Objectives: Heat shock protein 60 (hsp-60) and *C. pneumoniae* infection have both been associated with cardiovascular diseases, including abdominal aortic aneurysm (AAA). We examined whether there is any association between antibodies to human hsp-60 and anti-*C. pneumoniae* antibodies in AAA patients.
Material: Fifty-two AAA patients participated in the study. Thirty control subjects, matched for age and sex, without clinical signs and symptoms of cardiovascular and pulmonary disease took part in our study.
Methods: IgG and IgA antibodies to human hsp-60 were detected in sera samples using ELISA method and measured by means of internal units. In order to evaluate the level of IgG and IgA antibodies to *C. pneumoniae* microimmunofluorescence method was applied. *Chlamydia pneumoniae* Micro-IF test (Labsystems, Finland) was used according to manufacturer's instructions. The following criteria have been adopted to assess the type of infection:
1. chronic infection: IgG $\geq$1 : 128, IgA $\geq$1 : 32;
2. exacerbation of chronic infection (active infection): IgG $\geq$1 : 512, IgA $\geq$1 : 64
3. contact with the pathogen: IgG $<$1 : 128, IgA $\leq$1 : 8

Results: The mean concentrations of IgG and IgA antibodies to human hsp-60 were as follows:
IgG: AAA patients – 155.9 U/mL (range: 46.99–1001.0) healthy controls – 107.24 U/mL (range: 5.91–291.49)
IgA: AAA patients – 85.37 U/mL (range: 14.8–467.41) healthy controls – 61.85 U/mL (range: 0.0–216.78).
Percentages of AAA patients with serological markers of chronic and active *C. pneumoniae* infection or the contact with the pathogen were as follows: active infection – 32.69%, chronic infection – 53.85%, contact with the pathogen-13.46%. Mean concentrations of IgG and IgA antibodies to human hsp-60 in the three groups of AAA patients were as follows: active infection group: IgG – 238.76 U/mL; IgA – 87.58 U/mL; chronic infection group: IgG – 128.57 U/mL; IgA – 84.69 U/mL; contact with the pathogen group: IgG – 64.0 U/mL; IgA – 82.74 U/mL.
Conclusions: The concentrations of antibodies to human hsp-60, both IgG and IgA, were significantly higher in AAA patients than in healthy controls ($P < 0.05$). Strong correlation was observed between titers extent of IgG and IgA antibodies to *C. pneumoniae* and the concentration of IgG antibodies to human hsp-60 in AAA patients. There was no correlation between titers extent of IgG and IgA anti-*C. pneumoniae* and the concentration of IgA antibodies to human hsp-60 in AAA patients.

P683 Evidence of poliovirus-specific memory immunity in seronegative elderly people

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Objectives: There are doubts about the poliomyelitis protection of the Dutch born between 1925 and 1945 because of their small seroprevalences. They were ineligible for the inactivated poliovirus vaccine (IPV) vaccination introduced in 1957, and they may have escaped natural infections because of reduced poliovirus circulation. Waning natural or vaccine-induced immunity may be a factor. To guarantee high protection levels, we must determine whether low or undetectable antibody levels increase susceptibility to infection or whether memory immunity provides protection.
Methods: In a Dutch study in 1999, 429 of the 1847 elderly subjects screened for all three types of poliomyelitis antibodies were challenged with a standardized, oral dose of live-attenuated, monovalent poliovirus vaccine (type 1 or 3) and followed for 8 weeks. Systemic and local immune responses and virus excretion were compared for four groups: seronegativity for type 1 or type 3 polio, natural immunity (seropositivity for all types and no vaccination history), and IPV immunity (seropositivity for all types and documented vaccinations).
Results and conclusions: In the quick antibody response and absence of IgM, the authors see clear evidence of memory immunity in about one-third of the elderly population without detectable antibodies against type 1 poliovirus. No evidence of memory immunity was found for another third; and there is no clarity for the remainder. The evidence of memory immunity for type 3 poliovirus is 5%.

P684 Prevalence of Tetanus immunity in the Kocaeli Region, Turkey

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Objectives: To detect antibody levels against tetanus in the general population in Kocaeli region, and its relation to age and gender.
Methods: This study has been performed in Kocaeli University Hospital. After having received their informed consent, 5 mL of blood was taken from outpatients, ages over 20 years. Serum levels of antitetanus antibodies were detected by an enzyme-linked immunosorbent assay system (Genzyme Virotech GmbH, Germany). The procedure was performed as indicated by the manufacturer. Results were expressed in units based on a standard curve drawn by using control sera. The results were accepted as follows: $<$0.15 IU/mL = susceptible, 0.15–1.0 IU/mL = basic protection, $>$1.0 IU/mL = full protection.
Results: Overall, 25.7% of the population had full protective levels of tetanus antibodies ($>$1.0 IU/mL). Full protectivity rate was dropped with increasing age. In the age groups of 21–30, 31–40, 41–50, 51–60 and $>$61, full protectivity rates were 63, 43.7, 18.2, 8.5 and 2.8%, respectively. Full protectivity rates were similar in the male and female population (26.3, and 25.3%, respectively). In 55.6% of all outpatients, antibody levels were detected between 0.15 and 1.0 IU/mL accepted as basic protection level. In the 21–30, 31–40, 41–50, 51–60 and $>$61 age groups, basic protectivity rates were 32.6, 52.1, 59.1, 64.1 and 64.8, respectively. In 18.7% of all patients antibody levels were $<$0.15 IU/mL accepted as susceptible and at risk. In the 21–30, 31–40, 41–50, 51–60 and $>$61 age groups, susceptibility rates were 4.3, 4.2, 22.6, 27.3 and 32.3%, respectively. If 0.03 IU/mL is accepted as treshold value, all 592 tested outpatient were immune against tetanus.
Conclusions: Tetanus is a prime example of a serious preventable disease. Effective control requires immunizing every individual by vaccination. In Kocaeli region susceptibility to tetanus (antibody levels $<$0.15 IU/mL) increases significantly ($P < 0.001$) over than 41 years of age. These data suggest that routine booster immunization should be programmed every 10 years over than 40 years of age.

P685 Placental transfer of total immunoglobulin G and subclasses in a Turkish population

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Objectives: Maternal immunoglobulin G (IgG) transferred across the placenta to the fetus during intrauterine life. It is a remarkable component for newborn defense mechanism against infection. There are numerous publications on IgG subclass levels in different populations. Therefore, we investigated placental transfer of total IgG and IgG subclasses in a Turkish population.
Methods: The concentrations of total IgG and its four subclasses were studied in 103 pairs of maternal and cord blood samples from pregnancies of various gestational ages ranging from 32 to 42 weeks. Total IgG values were studied by

using the method of turbidimetric assay and IgG subclasses by radial immunodiffusion and the results were compared.
Results: The mean ratios of all IgG subclasses were positively correlated to gestational age. Total IgG values were found at cord blood samples greater than maternal serum samples (Table 1).

Table 1 Mean immunoglobulin levels at mother and cord blood samples and statistical differences

IgG subgroups	Maternal blood	Cord blood	*P*
IgG1	8.47 ± 2.63	10.08 ± 2.95	0.0001
IgG2	3.65 ± 1.45	2.96 ± 1.33	0.0001
IgG3	0.79 ± 0.33	0.75 ± 0.32	0.02
IgG4	0.45 ± 0.37	0.52 ± 0.44	0.004
Total IgG	11.56 ± 4.25	12.62 ± 4.87	0.003

The mean values of cord/maternal concentration ratios of IgG subclasses at 32–42 weeks gestational ages were as follows: IgG1 (1.19) >IgG3 (0.95) and IgG4 (1.15) >IgG2 (0.81).
Conclusion: It was established that the transfer of IgG1 and IgG4 rates were found to be significantly more capable than that of IgG2 and IgG3 rates were the least capably transferred from mother to fetus. The different results as to placental transfer of IgG subclasses in the literature might be due to different maternal IgG subclass serum levels in the populations studied.

P686 Serum neopterin levels in patients with brucellosis

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Objectives: Brucella species are facultative intracellular pathogens capable of surviving and replicating within phagocytes. Brucellae ingested by macrophages are not killed by polymorphonuclear leukocytes. Neopterin is released from monocytes and macrophages by stimulation with viruses and intracellular bacteria like *M. tuberculosis*. Neopterin can be used as a sensitive marker at cellular immunity. The purpose of this study was to evaluate the importance of neopterin levels for diagnosis, follow up and chronicity of brucellosis.
Methods: A total of 30 patients whose clinical findings and Brucella standard tube agglutination tests were positive were included in the study. Nineteen of the patients were male and 11 of them were female. The control group was composed of 30 healthy subjects (15 male, 15 female). The patients who had any infection, malignity and major surgical attempt were not included the study. The blood samples were collected from the patients under appropriate conditions, and centrifuged at 2000 r.p.m. for 20 min, and stored at $-20^{\circ}C$ immediately until anlyzed. Neopterin levels were measured by ELISA according to the protocol of manufacturer by Brahms Diagnostica GmbH (Berlin, Germany).
Results: Mean neopterin level was 54.15 ± 33.66 nmol/L at study groups and 8.75 ± 2.48 nmol/L at controls ($P < 0.001$). It was determined that the neopterin levels measured at application time of the patients had a linear correlation between the length of complaint ($r = 0.560$, $P = 0.001$, Spearman's rank test.). While the mean neopterin level at the patients whose complaints were more than 30 days was 79.09 ± 34.91 nmol/L, and it was 39.71 ± 23.41 nmol/L at the patients whose complaints fewer or equal to 30 days ($P = 0.002$). There were no correlation between neopterin levels with Brucella standard tube agglutination test, 2-mercaptoethanol test, erythrocyte sedimentation rate and C-reactive protein levels.
Conclusion: It was concluded that the neopterin levels are increased at patients with brucellosis and it will be a supportive marker for diagnosis. In addition it was thought that continuing high levels of neopterin after 30th days of the disease could be an early marker for determining the chronicity of the brucellosis.

P687 Anticardiolipin and Beta2-Glycoprotein I antibodies in Greek patients with chronic hepatitis C and B (Hbeag Negative) virus infection

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Aim of the study: To determine the prevalence of anticardiolipin (aCL) and antibeta2-glycoprotein I (antibeta2GPI) antibodies in Greek patients with chronic hepatitis C and chronic hepatitis B (HBeAg negative) virus infection.
Patients and methods: Fifty-one (51) consecutive patients with biopsy proven chronic hepatitis C and HCV RNA positive in serum were studied. In eight patients the histological diagnosis was cirrhosis (15.7%). Forty-one (41) patients are actually on treatment. Twenty-four (24) consecutive patients with biopsy proven chronic hepatitis B (HBeAg negative) and HBV DNA positive in serum were also studied. Ten (10) patients (41.7%) were cirrhotic and 20 are actually on treatment. IgG and IgM aCL and anti beta2GPI antibodies in serum samples before and at 6 months during treatment, were measured by ELISA. A wide spectrum of autoantibodies were also tested.
Results: In chronic hepatitis C patients, IgG-aCL antibodies were positive in 1/51 (2%). IgM antibeta2GPI antibodies were positive in another patient 1/51 (2%). ANA > 1/160 in 3/51 (6%), ASMA >1/80 in 13/51 (25%), RF > 100 IU/mL in 6/51 (12%) and anti-TPO > 100 IU/mL in 3/51 (6%) were also detected. In chronic hepatitis B patients, IgM-aCL antibodies were positive in 1/24 (4%). ANA > 1/160 in 1/24 (4%), ASMA > 1/80 in 11/24 (46%), RF > 100 IU/mL in 10/24 (41.7%) were also detected. No antibody reactivity has changed during treatment. The positive detection of aCL and antibeta2GPI antibodies was not correlated with any clinical manifestation.
Conclusion: The prevalence of aCL and antibeta2GPI antibodies in Greek patients with well characterized chronic hepatitis C and B is not significant and does not change during treatment. Their presence is not correlated with any clinical manifestation.

P688 HLA-B27 antigen and brucellosis

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Objectives: Osteoarticular involvement is a common complication of brucellosis including arthralgia, sacroileitis, spondylitis and arthritic episodes. Some of the symptoms resemble those associated with the reactive spondyloarthropathies, which are associated with an increased frequency of the histocompatibility antigen HLA-B27. There were few conflicting reports about association between osteoarticular brucellosis and HLA-B27. In this study, we aimed to find that whether the occurence of osteoarticular brucellosis could be predisposed by HLA-B27 antigen or not.
Methods: Seventy-eight patients with brucellosis followed up between April 1999 and April 2002 in our department, were included in this study. The mean of age the patients was 43 years (range: 15–73 years). Brucellosis was diagnosed with the following criteria: (i) clinical picture compatible with brucellosis and (ii) standard tube agglutination 1/160 titers and/or (iii) isolation of *Brucella* species from blood and/or bone marrow culture. Osteoarticular complications were considered to be present if there were obvious inflammatory signs in any peripheral joint, or unrelieved pain at rest in any deep joint with radiographic evidence of abnormalities. Detection of HLA-B27 antigen made by microlymphositic cytotoxicity method in peripheric blood. One hundred unrelated healthy individuals served as controls.
Results: Osteoarticular complications were defined in 25 of 78 patients (32.1%). The most observed complications were spondylitis (44%) and sacroiliitis (28%). The frequency of HLA-B27 was 14.1% (11/78) in all patients, while 8% in control group. It was 16% (4/25) in patients with osteoarticular brucellosis. Although HLA-B27 frequency in this group was slightly higher than those of others, there was no significant difference between patients with and without osteoarticular manifestations and healthy control group ($P > 0.05$).
Conclusion: We could not find any association between HLA-B27 antigen frequency and osteoarticular brucellosis.

P689 Ciprofloxacin, moxifloxacin and clarithromycin alter Th cell interferon gamma and interleukin-4 expression

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Objectives: Quinolone and macrolide antibiotics are routinely used for the treatment of critically ill patients with sepsis. These antibiotics modulate aspects of immune cell function [1,2]. Alteration in the profile of T helper cell cytokine expression will affect the Th1/Th2 profile and have knock-on effects on subsequent immune responses [3]. We examined the effect of increasing concentrations of ciprofloxacin, moxifloxacin and clarithromycin on Th cell cytokine expression.

Methods: Following ethical committee approval and informed consent, mononuclear cells were isolated from 20 healthy volunteers aged 20–48 years using single density gradient centrifugation. Cells were incubated for 4 h with ciprofloxacin (0–100 μg/mL), moxifloxacin (0–50 μg/mL) or clarithromycin (0–125 μg/mL) and stimulated with PMA/ionomycin in the presence of monensin. Cells were stained with antibodies to CD3 and CD4, prior to permeabilisation with saponin and intracellular staining with anti-IL-4 and anti-IFNγ, and flow cytometric analysis. Data are expressed as median percentage of CD3+/CD4+ and either IL-4 + or IFNγ + cells and were analyzed by Friedman analysis of variance with Wilcoxon signed ranks post hoc testing.
Results: Both moxifloxacin and ciprofloxacin dose dependently decreased IFNγ and IL-4 expression by Th cells (both $P < 0.0001$). Only IL-4 expression, however, was affected by clarithromycin ($P = 0.04$). There was no significant change in IFNγ/IL-4 expression (Th1/Th2 ratio) for moxifloxacin or ciprofloxacin, but the Th1/Th2 ratio increased with increasing concentrations of clarithromycin, from a median [range] of 5.3 [13–16.0] to 9.1 [1.8–18.8] ($P = 0.017$).
Conclusion: The quinolone antibiotics moxifloxacin and ciprofloxacin have pronounced effects on both Th1 and Th2 cytokine expression, without altering Th1/Th2 ratios. However clarithromycin decreased IL-4 but not IFNγ expression such that the Th1/Th2 ratio increased. Since a predominantly Th1 profile is considered favourable for resolution of acute infection, elucidation of the immunomodulatory profiles of antibiotics may in the future permit more rational antibiotic choice in critically ill patients.
Acknowledgement: We are grateful to Bayer Plc for financial support.

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P690 Cytokines and disseminated intravascular coagulation in sepsis

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Objectives: To investigate the relationships between tumor necrosis factor-alpha (TNF-alpha), interleukin (IL)-6, IL-10, transforming growth factor-beta (TGF-beta) and disseminated intravascular coagulation (DIC) in sepsis.
Patients: Methods: 30 patients (17 males, 13 females, mean age 69.8 ± 8.5 years) with severe sepsis (septic SIRS plus at least one organ dysfunction) were studied. The etiology of sepsis was: skin or joint infection ($n = 5$), pneumonia ($n = 10$), lung abscess ($n = 1$), pyelonephritis ($n = 6$), intra-abdominal infection ($n = 8$). The control group included 10 healthy subjects (six males, four females, mean age 66.5 ± 7.2 years). Serum cytokine levels were determined by using Elisa (Quantikine, R&D Systems, Minneapolis) on admission, on days 3, 10, 13 and on discharge.
Criteria for dic were: platelets $<150\,000/mm^3$, prothrombin time >15 s, fibrinogen <160 mg/dL, fibrin degradation products >10 μg/mL.
Results: Fifteen patients fulfilled the criteria for DIC. In DIC patients (group A) TNF-alpha levels were significantly higher than to non-DIC patients (group B) on admission [median (m) = 110.90; range (r) = 113.16 vs. m = 58.09; $r = 105.03$ pg/mL, $P = 0.022$] and on day 10 (m = 133.24; $r = 87.43$ vs. m = 44.89; $r = 89.27$, $P = 0.02$). We also found IL-6 levels significantly higher in group A compared with group B on admission (m = 214.28; $r = 380.94$ vs. m = 80.28; $r = 240.02$ pg/mL, $P = 0.045$) and on day 3 (m = 131.85; $r = 371.78$ vs. m = 64.20; $r = 154.02$, $P = 0.028$). On admission TGF-beta levels were detected significantly lower in DIC-group (m = 21 116.97; $r = 3477.46$ vs. m = 39 499.20; $r = 6838.90$ pg/mL, $P = 0.004$). No significant difference was found in IL-10 levels between the two groups. Logistic regression analysis demonstrated TNF-alpha levels on admission as sign prognostic factor for the development of DIC (odds ratio, 1:0001; 95% confidence intervals, 1.0000–1.0002, $P = 0.015$).
Conclusion: Pro-inflammatory cytokines, like TNF-alpha and IL-6, seem to be remarkable factors for development of DIC in sepsis. On the contrary TGF-beta may acts as an anticoagulant cytokine, reducing the effect of pro-inflammatory cytokines.

P691 Local and peripheral pro-inflammatory cytokine concentration in otitis media; possible *Mycoplasma pneumoniae* coinfection

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Background: Chronic otitis media with effusion (COME) often affects children. The development of COME is one of the most common consequences of the lack or inappropriate treatment of acute otitis media. COME is associated with retention of inflammatory mediators and cells in the middle ear cleft. Retention of effusion causes a change of an inactive mucoperiosteum in a secretory epithelium. It is associated with ongoing inflammation with the potential for pathologic changes and hearing loss. *Mycoplasma pneumoniae* (M.p) and *Chlamydia pneumoniae* (Ch.p), common pathogens of the human respiratory tract, have been reported to play an important role in the pathogenensis of many respiratory diseases including otitis media.
Objective: The aims of the study were to measure levels of inflammatory cytokines (IFN-γ, TNF-α and IL-8) in middle ear effusions (MEE) and in serum samples with respect to their reciprocal relations and their dependence upon degree of hearing loss and to evaluate serological markers of M.p. and Ch.p. infections in patients with COME.
Methods: Serum samples were obtained from 23 children (2–14 years of age) with COME. All patients suffered from hearing loss lasting 2–24 months. Concentrations of cytokines were measured by ELISA. This method was also used to detect IgM and IgG directed against M.p. IgG and IgA against Ch.p. were detected by MIF test.
Results: TNF-α and IFN-γ concentrations in MEE were comparable to their levels in serum. IL-8 concentration in MEE was significantly increased with respect to its concentration in serum (414 pg/mL-left ear, 314.1 pg/mL-right ear vs. 6.9 pg/mL in serum). Concentration of cytokines depending on the degree of hearing loss was compared. In children with hearing loss >20 dB levels of IL-8 and TNF-α in MEE were about twice higher in comparison with samples obtained from children with hearing loss <20 dB. Antibody titers consistent with Ch.p. infection were found in one child (4.3%). M.p. specific antibodies were detected in nine children (39.1%). The simultaneous presence of M.p. specific significant levels of IgG and IgM was detected in three children.
Conclusions: Persistently high levels of IL-8 in middle ear effusions suggest a close connection between IL-8 concentrations and the development and persistence of hearing loss. High incidence of M.p. specific antibodies suggests a possible contribution of this pathogen to the pathogenesis of COME.

P692 Do reactive nitrogen intermediates take part in elimination of *Staphylococcus aureus* during chronic staphylococcal infections of the upper respiratory tract?

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Objective: Chronic staphylococcal infections of upper respiratory tract constitute a common therapeutic problem and may indicate any defect in host defense. The persistence of infection may be connected with intracellular survival of *Staphylococcus aureus*. Nitric oxide and reactive nitrogen intermediates are reported to be important in the protection against *Staphylococcus aureus* and in host defense against intracellular pathogens. The aim of the study was to evaluate nitric oxide concentartions in plasma of patients with chronic staphylococcal infections of upper respiratory tract. In vitro studies were performed to assess capacity for NO production after stimulation of patient's blood by stimulus which had caused the infection.
Methods: The concentrations of nitrite in blood plasma of 19 patients with chronic staphylococcal infections of upper respiratory tract and 15 healthy volunteers were measured by colorometric method using Griess reaction. The synthesis of nitric oxide was activated in vitro in the samples of blood by incubation with staphylococcal strain isolated from swabs of patient's nose and throat or with model staphylococcal strain ATCC 25923 in control group. After induction the concentration of nitrite was measured in supernatant.
Results: Initial NO concentrations in group of patients were very varied (mean 20.99 μmol/L), whereas in control group the concentrations were

significantly lower (mean 0.06 μmol/L). After stimulation by staphylococcal strain NO concentrations have increased to small extent in group of patients, while in control group no changes were observed.
Conclusions: During chronic staphylococcal infection occurs elevated production of NO and increased readiness to NO production by leukocytes after stimulation in comparison to acute inflammatory response. It indicates possible participation of reactive nitrogen intermediates in organism defense during chronic staphylococcal infections as an auxiliary mechanism of oxygen burst.

P693 Long-term primary CD4 lymphocytopenia associated with spastic paraparesis and conjunctival microangiopathy. A rare syndrome of unknown pathogenesis and outcome

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Introduction: The syndrome of idiopathic CD4+ T-lymphocytopenia (ICTL) has been classified by both CDC and WHO as a distinct nosological feature: it was defined by a CD4 count <300 cells/μL and/or <20% of T cells, and a CD4:CD8 ratio <1, in absence of a known cause of immunodeficiency. A unique case report of a patient (p) with an ICTL of 10-year duration associated with an unexplained paraparesis and a conjunctival microangiopathy is reported and discussed on the ground of literature evidences.
Case report: A non-HIV-infected female p followed for a decade with an extremely severe ICTL (as expressed by a CD4 count of 8–25 cells/μL), and an isolated spastic paraparesis and a conjunctival ischemic microangiopathy is described. Despite a deep and prolonged immunodeficiency, no opportunistic disease (OD) occurred in a period of observation longer than ever reported. The paraparesis was diagnosed concurrently with ICTL, while ocular complication occurred 2 years later, but remained stable thereafter. Both disorders had no relevant changes during the subsequent 8 years. Notwithstanding an extensive workout, very limited immunologic abnormalities were detected (besides the extremely low CD4 count), and no apparent explaination was found for the disabling paraparesis. Repeated cranio-encephalic and lumbar spine TC, MRI, and PET scans proved normal, while a predominantly motor peripheral neuropathy was found. Autoimmune, endocrinologic, and collagen vascular disorders, Wegener's granulomatosis and Behçet disease were carefully excluded. Chemotaxis, phagocytosis and killing of neutrophils and macrophages did not show anomalies as well as in vitro lymphoproliferative assays and lymphocyte apoptosis.
Discussion: ICTL, whose pathogenesis warrants careful investigation, has been associated with a broad clinical spectrum, ranging from negligible or no disturbances, to severe lymphoproliferative disorders, OD, and other focal diseases, including neurological ones. Our case report of ICTL appears significantly different from all other literature reports. A decade follow-up was never described, and despite a CD4 count persistingly <25 cells/μL, OD never occurred.The prominent paraparesis was quoted one time only, while a ICTL-associated conjunctival microangiopathy was never described. The association of a long-lasting deep CD4 lymphocyte depletion in absence of any OD or neoplasm, and long-term isolated and stable paraparesis and conjunctival angiopathy, represents an absolutely exceptional finding.

P694 Pulsed-field gel electrophoresis demonstrated genetic diversity in a maxillary abscess of *Prevotella nigrescens*

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Objectives: Anaerobic infections like abscesses due to *Prevotella nigrescens* in previously sterile environments may be the result of ecological formation in dependence of the time. This must inevitably result in genetic rearrangements of the species. Therefore strain diversity of various colonies of *P. nigrescens* from two single infections was determined.
Methods: Pulsed field gel electrophoresis (PFGE) was applied to strains of *P. nigrescens* isolated from cultures of a patient with maxillary abscess and a patient with an acute peritonitis. Up to seven colonies from each culture plate were examined and compared with the typing results of arbitrarily primed PCR (AP-PCR). Coaggregation experiments were performed with these strains using a panel of Streptococci, including clinical strains of *S. mitis* and *S. bovis*, and the reference strains *S. parasanguinis* DSM 6778, *S. gordonii* DSM 6777, *S. sobrinus* DSM 20742, *S. salivarius* DSM 20560, and *S. mutans* DSM 20523 as coaggregation partners.
Results: The patterns of single strains of the maxillary abscess differed by more than three bands in the PFGE. These differences were not seen in the AP-PCR. Strains isolated from the patient with acute peritonitis demonstrated no differences in either method. Moderate coaggregation was seen between *S. parasanguinis* and *S. gordonae* and all strains of the maxillary abscess, but only three strains showed weak to moderate coaggregation with *S. mitis*. All strains isolated from the patient with peritonitis demonstrated strong coaggregation with *S. mitis*, *S. parasanguinis*, and with *S. bovis*.
Conclusions: Orofacial infections may be the result of chronic infection allowing strains to develop single genetic events. PFGE is more discriminative than AP-PCR to detect such events. The coaggregation may be of advantage in colonising compartments other than the oral cavity. The coaggregation of *P. nigrescens* isolated from the patient with peritonitis to *S. bovis* reflects the adaptation of this predominantly oral species to the intestinal flora.

Environmental epidemiology of fungi

P695 A survey on pathogenic fungi in potted plants from a hospital environment – Sari, Iran

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Objectives: The aim of this study was to analyze the soil collected from the potted plants in the hospitals for pathogenic fungi.
Methods: A total of 23 soil samples of potted plants were collected from Sari hospitals. Each sample contained about 200 g of soil, taken from a 0–10 cm depth. Then, samples were analyzed by two different methods: (1) About 50 g of samples were suspended in distilled water and were shaken vigorously for 10 min at room temperature, and allowed to settle for another 10 min. Supernatant was inoculated on to the Sabouraud's dextrose agar (S) supplemented with chloramphenicol (SC) and chloramphenicol and cycloheximide (SCC), and plates were incubated at room temperature for 4 weeks. During this period, the grown fungi was identified by macroscopic and microscopic characterization. (2) The keratin-baiting technique was used for isolation of keratinophilic fungi; therefore, approximately 50 g of each samples was placed into empty sterile Petri dishes, mixed with sterilized hair, and cut into small pieces. The presence of keratinophilic fungi was confirmed by low-power microscopic examination. Fragments on colonized hair were inoculated on to see and incubate for 2 weeks at room temperature.
Results: In one method, 100% of plates were positive for fungal growth and a total of 1150 colonies with 13 different type of fungi were isolated. *Penicillium* spp. (52.35%), *Paecilomyces* spp. (11.91%), *Cladsporium* spp. (3.74%), and *Aspergillus* spp. (3.13%) were predominant fungi. *Rhizopus* spp. was the uncommon. In this study, *Microsporum gepseum*, *M. cookei*, and *Chrysosporium* spp. were identified as keratinophilic fungi.
Conclusion: The presence of pathogenic fungi such as *Cladosporium*, *Aspergillus*, *M. gepseum*, and *M. cookei* in hospitals that isolated from this study are risk factor for patients.

P696 Is drinking water a potential vehicle for community-acquired aspergillosis? A study of water-borne *Aspergillus* species in the Province of Madrid

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Objective: The presence of conidia in bathrooms has recently been reported as a potential vehicle of spores causing community or nosocomially acquired invasive aspergillosis. The aim of our study was to determine the presence of

Aspergillus spp. spores in drinking water during different seasons across the province of Madrid (Spain).
Methods: We collected samples of drinking water from 37 taps across the province of Madrid where water is collected from 14 water reservoirs located in the mountains. Twelve stations treat the water and turn crude water into drinking water. From here, drinking water is kept in 22 deposits distributed throughout the Province, and these provide water for every dwelling in the Province. The selected taps were as follows: 19 taps located at the private residences (bathrooms and kitchens) of workers in our laboratory, 11 from fountains (10 of drinking water and 1 from an ornamental fountain) located in city squares, 5 were from the bathroom (3 located at pubs and 2 from petrol stations), and the last 2 located in two different laboratories. Every tap was tested twice, in August (summer) and in November (autumn), to obtain a total of 74 water samples. The samples were collected after letting the water run for 5 min to clean stagnant water from the pipe. We took 500 mL of water per sample, each of which was transported in sterile vacuums, and these were kept at 4°C until processing. Each sample was filtered by means of Millipore filters (Microfil TM V, 0.45 mm diameter pore). The filters were removed from the filtration structure and put over the surface of Sabouraud-chloramphenicol agar plates and incubated at 35°C for 7 days. The plates were checked daily to observe the growth of fungi.
Results: None of drinking water samples were positive for *Aspergillus* spp. either in summer or in autumn. The only positive sample was the water obtained from the ornamental fountain, showing growth for *Aspergillus niger* and dematiaceous fungi.
Conclusions: Although water-borne *Aspergillus* spp. conidia transmission has recently been reported, we did not find any samples in drinking water, and only one positive sample at a non-drinking water point was found. The deposits in the Province are exposed to air (a main source of conidia), and we did not find the presence of spores in any selected tap. Our data did not support the idea of the presence of conidia in drinking water as a potential vehicle of spores causing invasive aspergillosis.

P697 Public awareness of the risks and spread of fungal infections

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Objectives: Control of fungal infections in women, especially young mothers and teenagers.
Methods: A survey carried out in May 2002 by community health workers in Uganda Makindye division indicates that 80% of women and pregnant mothers in this area have the problem of fungal infections, especially candiasis. Bukasa development group have gone around sensitizing women and reassuring them that fungal infections are common and are not acquired through sexual relations and that they should avoid using dirty and contaminated toilets. We advised them to stop decieving health workers when they go to clinics; they must tell the truth so that they can get the right medications and doses. They should not fear to go to clinics to be checked and advised by doctors. We teach them how to handle their underware and other clothes by washing with soap, drying them in sunshine, and ironing them before use, they should keep their bodies and private parts clean and dry.
Results: Women, especially young mothers and teenagers, have started coming to our clinics and they explain their difficulties with confidence. This has helped the medical workers to find out the different types of fungal infections and how to treat them.
Conclusions: The rate of fungal infections can be decreased if many projects come out to sensitize the community about this problem. Even local leaders and the press should keep on advertising and encouraging the people so that even those who are infected and are fearing to be known should come out and get treated.

P698 Dermatophyte fungi contaminations in 16 public bathrooms in Golpayegan, Iran

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It has been established that bathrooms contribute to the spread of dermatophytosis in susceptible hosts. The recent studies indicate that the infection of dermatophytosis is increasing in different cities of Iran, but the species of the fungal agent in each city is different. The fungal contamination of 16 indoor public bathrooms was investigated by the method of carpet sampling. Two hundred and twenty-four samples from the floor, slippers, dressing rooms, and related areas of each bathroom were tested for the presence of dermatophyte fungi in different places of the city. In this investigation, different dermatophytes such as *Trichopyton rubrum* and *T. mentagrophytes* were observed in very low level, but the most common dermatophyte recovered was *Microsporum gypseum*. *M. gypseum* is a geophilic dermatophyte and it seems that the reason of high-level spreading of this fungus in the city is because of materials that are used in bathroom buildings (clay and straw) and special climate conditions of Golpayegan. The fungal contamination of these bathrooms under specific conditions could be an alarm for induction of dermatophytosis in compromised and susceptible hosts.

Nosocomial infections

P699 Vaginal colonization in patients before gynecological procedures

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Objective: Analysis of vaginal flora in women with different diseases, without previous antibiotics, before different gynecological operations.
Methods: We studied microbiological results of vaginal swabs from 62 patients aged 22–83 years, admitted to a three tertiary care hospitals. Samples were collected in operating room, before performing povidone–iodine (Betadine–Egis) antisepsis procedures. Isolation and identification were performed by standard methods and VITEK system (Biomerieux).
Results: We examined 62 women with: Ca corporis uteri, 8; Ca colli uteri, 14; Myomata uteri, 15; Ca ovariorum, 9; Cystis ovarii, 9; Tu vaginae, Sterilitas, 2; Graviditas extrauterina, 1; Descensus uteri, 3. *Lactobacillus* sp. was isolated in 38 cases (61% women) and *S. epidermidis* in 25–40%. Other isolated microorganisms were: *Escherichia coli* – 32 (52%) patients, *Enterococcus faecalis* – 29 (45%), *Streptococcus agalactiae* – 17 (27%), *Gardnerella vaginalis* – 13 (21%), *Staphylococcus aureus* – 8 (11.2%), *Candida albicans* – 14 (22.5%), *Ureaplasma urealyticum* – 15 (24%), *Mycoplasma hominis* – 3, *Prevotella* spp. – 8, *Peptostreptococcus* spp. – 7, and 14 other isolates.
Conclusions: In such a group of women, the natural vaginal microflora is changed, perhaps due to underlying disease. *Lactobacillus* sp. was present in only 61% examined women. Other isolated bacteria should be considered as potential causes of postoperative infections.

P700 Postoperative infections caused by mycoplasma in patients after gynecological procedures

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Objectives: To characterize the infections caused by *Ureaplasma urealyticum* (U.u.) and *Mycolasma hominis* (M.h.) in patients after gynecological operations.
Methods: During 1 year, there were four patients with signs of infection after caesarean section and negative standard aerobical, anaerobical, and mycological cultures. Wound exudates, vaginal swabs, and urine were cultured on A7 medium and Mycoplasma IST test (Biomerieux) which detects the presence of Mycoplasms and determines their antimicrobial susceptibility. A7 cultures were incubated in 37°C in microaerofilic bags for 48 h.
Results: Both A7 and Mycoplasma IST tests were positive in four patients. The culture results are presented below in the Table 1.

Table 1

	Wound		Vaginal swab		Urine	
Patient No.	M.h.	U.u.	M.h.	U.u.	M.h.	U.u.
1	+++	-	++	-	-	-
2	+++	+++	+++	+++	-	-
3	++	-	+++	-	<104	-
4	++	++	+++	+	>104	<104

Conclusions: *M. hominis* and *U. urealyticum* are capable of causing serious infections in patients after gynecological procedures. In case of negative standard cultures, the presence of mycoplasma should be suspected and appropriate media inoculated.

P701 Immunobiological preparations in complex of treatment and sanitary measures in obstetric practice

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Objectives: The active introduction of a new medical technology in the obstetrical pediatric practice such as using a new forms of the immunobiological preparations being developed on the basis of studying natural mechanisms of the human organism protection is carried out for perfecting perinatal care for the women who are going to have babies, pregnant women, and their neonates from the risk group of the infectional pathology.
Methods: The newly developed immunoglobulin preparation with the monomeric form of IgG features the pronounced treatment and prophylactic action as it permits a prolonged circulation of IgG in the blood channel to be achieved. The selection of production-perspective bacterium strains the representatives of the obligate human microflora on the basis of studying their antagonistic activity to pathogenic and conditioned pathogenic microorganisms, adhesivneness, and resistence taking into account biotechnological characteristics (rate of increasing biomass, duration of storage and, etc.) permits a new manycomponent eubiotic from lacto- and bifidobacteria on nonallergic basis to be developed.
Results: The immunoglobulin for intravenous injection has been included in the standard complex of antibactericides for the prophylaxis of the possiblity of infecting a women in child birth in the case of premature birth with tearing amnion membranes at temporizing tactics and also for the prevention of developing and generalizating the infectious pathology in premature infants and neonates with the low birth weight. The normaflora preparations in general use for the women who are going to have babies, pregnant women, the women in childbirth, and neonates depending upon the character of recognized disorders in their microfloras. Eubiotics being developed from lactobacilli are preferable for vaginomicroflora disorders.
Conclusions: The use of a new form of imunobiological preparation permits reduction of the infectious morbidity in perinatal stage to be achieved.

P702 Urinary pathogens and their in vitro susceptibility to antibiotics

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Objectives: To determine the susceptibility of urinary pathogens isolated from semiquantitative cultures toward common antibiotics.
Methods: Over 2 years, we tested the susceptibility of 1072 bacterial strains isolated from urine specimens of inpatients to the most common antimicrobial agents. The susceptibility test was determined by the agar disk diffusion method.
Results: *Coli* forms remained the commonest pathogen, being (64.1%) of isolates. Second most common was *Enterococcus* spp. (18%) and it was closely followed by *Pseudomonas* spp. (12.2%). Coagulase-negative staphylococci and *Staphylococcus aureus* were the other main pathogens which remained at 3.3 and 2.2%, respectively. In terms of percentage sensitivities of *E. coli* to antibiotics, ciprofloxacin amoxicillin/clavulanic acid and amikacin covered the highest proportion of pathogens at 87, 80, and 87%, respectively. *Enterococcus* strains were 80% sensitive to amoxicillin/clavulanic acid and 100% sensitive to glycopeptides. *Pseudomonas aeruginosa* strains were sensitive to imipenem (80%), ciprofloxacin (65%), and amikacin (80%).
Conclusion: *E. coli* was identified as the chief causative agent of urinary tract infections. Monobacterial infection was observed in the majority of cases. Quinolone beta lactame and aminoglycoside antibiotics remain the first choice of U.T.I.

P703 Monitoring of bacterial origin systemic inflammatory response syndrome marker procalcitonin in cardiac surgery with cardiopulmonary bypass

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Objectives: To study the correlation between manifestation of systemic inflammatory response syndrome (SIRS) symptoms and concentration of procalcitonin (PCT) in blood plasma in perioperative period in cardiac surgery – the analysis of results of the first experience.
Methods: Fifteen high-risk patients with the acquired heart diseases before and after surgery with cardiopulmonary bypass (CPB) were enrolled in the study (mean age 50 ± 9.8 years). Eighty-one assays of PCT were made using the immunoluminometric method (PCT LumiTEST BRAHMS Diagnostica GmbH, luminometer Lumat LB 9507 Berthold Technologies GmbH). SIRS was defined according to the classification of the Society of Critical Care Medicine Consensus Conference, 1997.
Results: There were no cases of SIRS before surgery; PCT concentration did not exceed 0.5 ng/mL (in average 0.32 ± 0.05 ng/mL). Following SIRS manifestations during the first 24 h after surgery, the patients were divided into two groups: group I – without SIRS, group II – with SIRS. Serial PCT tests in early postoperative period on 2nd, 3rd, and 6th days after surgery demonstrated faster decrease and normalization of the PCT concentration in blood plasma of patients of group I in comparison with the group II. There were no postoperative complications in group I. In group II PCT concentrations were higher and remained elevated for a longer period of time than in group I. Three patients from group II developed lethal infectious complications in early postoperative period: two cases of multiorgan failure (MOF) and one case of sepsis. There was no significant difference in APACHE II scores between the groups with and without SIRS during the first day after surgery (7 ± 2 in the first vs. 9 ± 3 in the second group). This difference in APACHE II scores between the two groups appeared later following the development of postoperative complications (Table 1).

Table 1 PCT concentration measurements (ng/mL)

Group	Postoperative complications	*n*	Day 1	Day 2	Day 3	Day 6
I (no SIRS)	No	8	0.81 ± 0.35	0.69 ± 0.27	0.56 ± 0.15	0.37 ± 0.22
	MOF	2	7.52 ± 1.43	7.89 ± 3.87	5.80 ± 1.19	6.35 ± 2.5
II (SIRS)	Other complications	5	8.14 ± 7.07	5.96 ± 5.27	6.41 ± 7.19	0.63 ± 0.27

Conclusions: The first experience of monitoring PCT concentration during the early postoperative period has revealed the marker's informative and prognostic importance in cardiac surgery practice.

P704 Infections after open-heart surgery

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Objective: To know the rates of NI (nosocomial infection) in patients operated on open-heart surgery.
Patients and methods: The 670 patients (pts) operated on open-heart surgery in our hospital from January 2000 to November 2002 were included. Preoperative protocol regarding infection control includes: shower with 4% clrohexidine soap the night before surgery and again repeated the day of surgery. Hair removal immediately before surgery by clipping.
Antibiotic prophylaxis: Cefuroxime 1.5 g. IV/8 h. All the pt are prospectively studied since the day they are operated until the end of the episode. A computer based surveillance system INOZ is used during admission and continued 1 years after discharge. CDC definitions of nosocomial infection and NNIS (National Nosocomial Infection Surveillance) score are used.
Results: Age and sex: 461 (68.8%) men, mean age 66.2 years (SD 10.8). Mean preoperative stay in the hospital 6.2 days (SD 10.4). NNIS score 0, 34 pts; score 1, 450 pts; score 2, 181 pts, and score 3, 5 pts. Infections at admission: 40 pts (20 urinary tract, 7 respiratory, 2 skin. Nosocomial infections: 144 pts (21.49%) acquired 197 NI, 35 of them were surgical site infections (SSI): 12

incisional superficial, 10 deep incisional, and 13 organ space. NNIS score 0, 0%; 1, 4.2%; 2, 7.2%, and 3, 40%. Microorganisms in SSI: *S. aureus* 5, CNS 11, polymicrobial 5, *S. marcescens* 1, *P. aeruginosa* 1, Propionibacterium 1. Urinary tract infections (UTI): 53 pts. Etiology: *E. coli* (17), *E. faecalis* (11), *C. albicans* (4), *Serratia* (4), and *P. aeruginosa* (3) were the most frequent etiological agents. Respiratory infections: 57 pts. Pneumonia: 8 cases. Bacteremia: 17 cases: 3 catheter related, 6 primary, and 8 secondary bacteremias. Microorganisms: *E. coli* (4), *S. marcescens* (4), *S. aureus* (4), *P. aeruginosa* (1), *S. epidermidis* (1), *Candida* spp. (1), *E. cloacae* (1), and *Corynebacterium* spp. (1).
Conclusions: Nosocomial infections other than SSI are the most frequent (33% respiratory and 26.9% urinary) infections. Oral clorhexidine rinses are now being used to decrease the overall incidence of nosocomial respiratory infections according to the CDC guideline for prevention of nosocomial pneumonia along with an effort directed to decrease the time on mechanical ventilation, and a preoperative urine culture to decrease UTI.

P705 The in vitro comparative activity of old and new quinolones against bacterial isolates from surgical wounds

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Objectives: The aim of this study was to determine the range of pathogens in wound swabs, to ascertain the in vitro susceptibility of the isolated bacteria, and to assess the value of quinolones for clinical use.
Methods: Over a year, we tested the susceptibility of 502 bacterial strains isolated from confirmed wound infection specimens. For the identification of these bacteria the Api system was used. The susceptibility to antibiotics was determined by the agar disc diffusion method.
Results: The Gram-negative bacterial strains, 288 (57.1%) predominated over Gram-positive ones, 214 (42.9%).The distribution of Gram-negative into different taxons indicates a clear prevalence of enterobacteriaceae (33%) over *Pseudomonas* (17.9%) and *Acinetobacter* (7.5%). *S. aureus* (20.7%) predominated among Gram-positive bacteria followed by coagulase-negative staphylococci (14%) and enterococci (7.7%). The susceptibility of the most commonly isolated bacteria to ciprofloxacin and moxifloxacin were: *E. coli* (79 and 95%), *P. aeruginosa* (65% and 70%), *S. aureus* (55 and 60%), and C.N.S. (29 and 83%), respectively.
Conclusion: These results indicate that moxifloxacin is slightly less active than ciprofloxacin thus both quinolones provide superior in vitro activity in wound pathogens compared with usually employed autibiotics.

P706 Cefuroxime prophylaxis is effective in herniated disk surgery: a double-blind, placebo-controlled study

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Objective: To assess the efficacy of one single, preoperative, 1.5 g dose of cefuroxime in preventing surgical site infection after surgery for herniated disk.
Methods: Patients >18 years undergoing surgery for herniated disk at two university hospitals from April 1994 to March 2000 were included in a double-blind, placebo-controlled trial. Surveillance of postoperative infections was conducted according to criteria derived from the Centers for Diseases Control and Prevention (Garner et al. 1988) and from a study on antibiotic prophylaxis in clean surgery (Platt et al. 1990). Standardized information on signs and symptoms of infection were collected during hospital stay and at a 6-week follow-up visit by the surgeon, and through phone calls at 3 and 6 months.
Results: Patients allocated to cefuroxime ($N=613$) or placebo ($N=624$) were similar with respect to demographic characteristics, preoperative hospital stay, number of comorbidities, and type and duration of surgery. Eight (1.3%) patients in the cefuroxime group and 18 patients (2.9%) in the placebo group developed a surgical site infection (adjusted OR 2.39; 95% CI: 1.02–5.58; $P=0.044$). A diagnosis of spondylodiscitis was made in 9 patients in the placebo group, but in none in the cefuroxime group ($P=0.004$). Infection was diagnosed during hospital stay in 5 patients in the placebo group (two spondylodiscitis, three wound infections), while all the infections in the cefuroxime group were diagnosed after hospital discharge. Infection was microbiologically documented in 12 patients in the 2 groups: 11 were caused by Gram-positive organisms (*S. aureus*: 3; coagulase-negative staphylococci: 5; *P. acnes*: 2; *P. acnes*, and coagulase-negative staphylococci: 1).
Conclusions: A single, preoperative dose of cefuroxime significantly reduced the risk for surgical site infection, most notably spondylodiscitis, occurring after surgery for herniated disk.

P707 Incidence of the site operative infection in orthopaedic surgery

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Objective: To evaluate the incidence of the site operative infection in two units of the orthopedic surgery of our hospital.
Methods: All the patients admitted in the two orthopedic surgery units for a first operation and whom stayed more than 48 h in the hospital were recruited for this study which duration was 3 months. The operative wounds were controled regularly. A swabbing out was done when a discharge appeared on the wound and addressed immediately to our laboratory. The samples were tested. The strains isolated were identified and a susceptibility test according to NCCLS determined.
Results: Two hundred and thirty patients were hospitalized during the study period, but only 180 patients were recruited. The sexe ratio was 6.64 in the unit A and 3.14 in the unit B. The male patients were younger (the mean age was 35.5 years) than the female patients (the mean age was 49.4 years). The number of surgeries according to NRCA classificaion was 23 (class I), 118 (II), 20 (III), 16 (IV), and 3 (V).The infection rate was equal to 0% for the classes I, III, and Vand 3.77 and 1.53% for the class II, respectively, in the units A and B, and 22.22 and 14.28% for the class IV, respectively, in the units A and B. Six patients out of 180 were infected (3.33%), five were males and four were young (<30 years old.).The sex ratio of the infected and non infected patients was nearly the same. The preoperative hospitalization duration was 10.25 days for the infected patients and 6.4 days for the non-infected patients. We did not find a significant difference between the urgent operations (3.7%) and the programmed operations (3.33%), but a significant difference between the major surgeons infection rates (3.33–4%) and the young surgeons infection rates (6.6–16.66%). Ten specimens out of 16 tested were positive. Seven specimens out of 10 were monomicrobial. Thirteen strains were isolated and the main agent was *Staphylococcus aureus*: 91% (7 out of 13). Two *S. aureus* out of three were MRSA in the unit A.
Conclusion: The incidence of the site operative infection in our study was 3.33%. It obvious that this rate is may be under the real infection rate because of the patients with implants. These patients must be controled 1 year later. The duration of the preoperative hospitalisation and the insuffiscent experience of the surgeons were the two major risk factors found in our study. *S. aureus* is the most frequently isolated agent in the two units. In the unit A we isolated 2 MRSA, so measures must be taken to limit its diffusion.

P708 Outbreak of nosocomial aspergillosis in a general medical ward

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Background: Two outbreaks caused by *Aspergillus* spp. were detected recently in our hospital. The first one was related to renovation activities undertaken in our hospital general medical ward consisting of fixing, tiling and updating the furniture of the infirmary area in November 2001. The second one was related to street building works taken place close to the hospital, in July 2002.
Objectives: To describe prospective data collected from February to December 2002, from 34 patients in whom nosocomial bronchial colonization (NBCA) or probable nosocomial invasive pulmonary aspergillosis (PNIPA) was diagnosed. To study control measures and results concerning aerial contamination.
Results: During the mentioned period, 12 cases of PNIPA were diagnosed, another 21 were classified as NBCA and one could not be determined (1.1% of admissions to our medical ward). *A. fumigatus* (21), *A. flavus* (13), and five terreus were found. The first outbreak originated 29 cases and 5 cases the second. 22 of them had been admitted between November and February, before the outbreak was detected. Another patient was in hospital while street works were going on. Admission was due to acute respiratiry failure, infection or pneumonia. Median age was 73.82 years. PNIPA cases median hospital stay was 36.45 days (vs. 9.5 days for Chonic Obstructive Pulmonary Disease patients (COPD)). All but six patients showed other bacteria or fungi growth in their sputum. All but tree suffered from severe COPD, asthma, pulmonary

fibrosis or bronchiectasis and were on chronic corticosteroid therapy. None of them was neutropenic. PNIPA patients were treated with amphotericin B. Colonized patients were treated with itraconazole if discharged on oral corticosteroid therapy. 33.3% of PNIPA and 27.7% of NBCA died. It is difficult to demonstrate that death was the result of aspergillosis but was probably a contributing factor in some cases. Patients were moved, as soon as possible, to another hospital facility and a thorough cleaning and repairing procedure was undertaken. Colony counts per 1000 L of filtered air varied in patients' rooms but were usually higher in the corresponding shower facility and diminished to 0–2 cfu/1000 L afterwards.
Conclusion: As has been shown lately, severe COPD and corticosteroid treatment are important risk factors for *Aspergillus* infection. Environmental disturbances together with humidity and poor shower facility maintenance were the probable source of the outbreak.

P709 Recurrent endophthalmitis due to *Enterococcus faecalis*: the bacterium was localized in the capsular bag

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Introduction: Postoperative endophthalmitis is a rare, albeit serious, complication of intraocular surgery. The most frequent isolated bacteria are coagulase-negative staphylococci, *Staphylococcus aureus*, streptococci, miscellaneous Gram-positive organisms and Gram-negative organisms.
Case: We report the case of a 85-year-old man with a history of diabetes mellitus, who developed a recurrent endophthalmitis caused by *Enterococcus faecalis* following cataract surgery. Nine days after phacoemulsification cataract extraction, the patient developed acute endophthalmitis. Aqueous humour and vitreous were collected. Direct examination of the samples after Gram staining revealed Gram-positive cocci arranged in short chains only for the vitreous specimen. Cultures under aerobic and anaerobic conditions yielded *E. faecalis*. The patient received intravitreal vancomycin and ceftazidime, and intravenous vancomycin for 8 days followed by 10 days of piperacillin. A second intravitreal injection of vancomycin was performed 4 days after the first one because there was no clinical improvement. Associated topical treatment included ticarcillin, vancomycin and gentamicin. Four months after the onset of the infection, the persistence of severe intraocular inflammation led to a later enucleation. Bacterial cultures were performed on aqueous humour, vitreous and capsular bag. Only the capsular bag was positive for *E. faecalis*. Scanning and transmission electron microscopy allowed the discovery of adherent bacteria to the posterior capsule and in intramacrophagic localization.
Conclusion: This case shows that adhesion factors are also important virulence factors for *E. faecalis* endophthalmitis, in addition to cytolysin which is the major virulence factor. The antibiotherapy failure is explained by the fact that the bacterium was sequestered in the posterior capsule, escaping total eradication and thus producing recurrent endophthalmitis. Our report also underlines the contribution of electron microscopy (several descriptive pictures) showing the exact localization of *E. faecalis* and the massive colonization of the capsular bag by the bacterium.

P710 Detection of *P. carinii* in sputum of asymptomatic HIV-seronegative oncologic patients

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Objectives: To investigate the presence of asymptomatic carriers of *P. carinii* among oncologic patients without the acquired immunodeficiency syndrome.
Methods: Induced sputum specimens from 304 consecutive oncologic patients (130 M, 174 F, of mean age 56 years), were studied for the detection of *P. carinii* cysts and trophozoites using a direct immunofluorescent procedure. Any specimen which contained at least two typical cysts considered positive for the presence of *P. carinii*. All patients were HIV seronegative and had no evidence of pulmonary infection. Hematologic malignancy in 176 (57.9%) and solid tumors in 128 (42.1%) of our patients were the underlying malignancies. 225 out of the 304 patients were undergoing chemotherapy during the last six months.
Results: *P. carinii* cysts and trophozoites were detected in 68 out of 304 of our patients (22.4%). There was no significant difference between the patients with hematologic malignancy (23.2%) and those with solid tumors (19.5%). All 68 of our patients with presence of *P. carinii* in their sputum had undergone chemotherapy during the last six months and 58 of them (85.3%) were also received long-term therapy with corticosteroids at the time of diagnosis (mean duration 3 months).
Conclusion: The prevalence of *P. carinii* colonization is quite high among oncologic patients. It is possible that the dysfunction of the immune response due to chemotherapy and long-term treatment with corticosteroids in these patients provide this colonization during which the organism persists in a latent state. This may imply a higher risk of pneumoniae in these patients due to reactivation of the organism.

P711 Influenza outbreak on a hematological ward

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Objectives and methods: To describe an influenza outbreak on a hematology ward. In March 2002, at the hematological ward of our hospital, virological cultures from a patient with symptoms of respiratory infection, revealed influenza A-virus. The patient was treated with zanamavir and respirotory isolation precautions were implemented to prevent spread; furthermore contact-patients were nursed in isolation. At the end of that week it became clear that 14 health care workers from the ward were at sick-leave simultaneously. Ten out of these health care workers experienced symptoms of influenza and were moderately ill. Soon, virological cultures from one of the contact-patients was positive with influenza A-virus. As a third positive patient was identified, an additional PCR-detection for influenza for all respiratory samples from this ward was performed for the fastest identification of new positive patients. The hygienic precautions were intensified; positive patients were nursed in cohort-isolation, contact patients were nursed in a separate cohort and received zanamavir prophylaxis, and new patients were nursed in protective isolation. In 3 weeks, 13 patients were positive for influenza. From these, six patients were positive in PCR and in conventional cell culturing, four patients were detected by PCR only, for 3 patients cultures were positive but no PCR was performed. From literature, we expected the clinical picture of our immunocompromised patients to be more severe, however, the clinical picture of the health care workers, who were moderately ill with a sick-leave of 1–2 weeks, was much more severe than the patients who merely experienced mild symptoms such as rhinitis and somewhat elevated temperature. Possibly, the prompt administration of zanamivir ameliorated the clinical picture in the patients.
Conclusion: In the influenza-outbreak we encountered at our hematology ward, a causative relation with health care workers was very suggestive. A total of 13 patients were positive, however, due to the high sensitivity of the PCR, we identified more positive patients then before PCR detection was implemented, and to our relief no major illness was encountered. Despite recommendations of, i.e. the CDC and the WHO, the Dutch guidelines do not advocate influenza vaccination of health care workers. In our hospital, the board of directors has recently approved the vaccination of health care workers that take care of haematological patients.

P712 Successful pre-exposure hepatitis B virus vaccine prophylaxis for hospital personnel

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Objectives: Nosocomial infection among hospital personnel by hepatitis viruses, especially HBV, has been well documented. The aim of this study was to investigate the prevalence of HAV, HBV, HCV and HTLV-1 infection among hospital personnel and to develop means to protect them from HBV infection.
Methods: All hospital personnel of four Prefectural hospitals in Miyazaki prefecture, Japan were screened for HBc, HBs, HCV, HAV, HTLV-1 antibodies, and HBsAg in 1980, 1988, 1997, and 2002. All anti-HBs negative personnel were vaccinated with HB vaccine. Those who lost antibody protection again received HB vaccine at 5–8-year intervals. Each year, newly recruited personnel were screened for the same virus markers and vaccinated with HB vaccine.
Results: All virus markers tested decreased from 1980 to 2002. HBsAg from 3.5% to 2.0%, anti-HBc from 36.8% to 19.6%, anti-HCV from 2.3% to 0.6%,

anti-HA from 38.7% to 10.2%, and anti-HTLV-1 from 6.3% to 3.2%. The anti-HBs prevalence gradually increased after the initiation in 1988 of HB vaccine, 23.3% in 1980, 34.9% in 1988, 77.3% in 1997 and 81.7% in 2002. Acute hepatitis B was diagnosed in 4 nurses between 1980 and 1988, after which no hepatitis B cases were found. During the observation period, one administrative clerk developed acute hepatitis A.

Conclusion: HAV, HCV and HTLV-1 infection have decreased in recent years, with the decreases in prevalence seen in the general population reflected in lower rates of nosocomial infection. However, HBV infection remains relatively high in the general opulation of Japan. Vaccination was effective in preventing the spread of hepatitis B among Japanese hospital personnel.

Epidemiology of resistance 2

P713 Species distribution and antifungal susceptibility patterns for *Candida* isolates from the SENTRY Antimicrobial Surveillance Program (Europe)

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Objectives: Susceptibility of *Candida* species should be monitored against azole compounds. *Candida* species isolated from bloodstream infections (BSI) during 1999–2001 were compared with potentially pathogenic strains of *Candida* species isolated from other body sites (Other).
Methods: A total of 469 strains were collected by 19 tertiary care centers in Europe (12 countries including Turkey and Israel). At the reference laboratory, confirmation of identification and broth microdilution susceptibility testing were performed (NCCLS M27 for azoles and 5-flucytosine, *E*-test for amphotericin B).
Results: Overall, *C. albicans* (Ca) represented 64% of all isolates, followed by *C. glabrata* (Cg) (15%), *C. tropicalis* (Ct) (10%), *C. parapsilosis* (Cp) (5%). *C. krusei* represented only 1%. MIC90s (mg/L) to fluconazole (Flu), itraconazole (Itra), ravuconazole (Ravu), voriconazole (Vori), 5-flucytosine (5FC), and amphotericin B (AmB) are shown below:

Site/Species (*n*)	Flu	Itra	Ravu	Vori	5FC	AmB
BSI (181)						
Ca (112)	0.25	0.06	0.015	0.015	0.25	1.5
Cg (23)	32	2	1	0.5	0.12	4
Cp (19)	2	0.25	0.03	0.015	0.25	4
Ct (16)	2	0.5	0.12	0.06	128	2
Others (288)						
Ca (187)	0.25	0.12	0.007	0.015	0.5	0.75
Cg (47)	16	2	0.5	0.25	0.12	2
Cp (6)	1	0.5	0.06	n.a.	0.12	2
Ct (29)	2	0.5	0.12	0.06	128	2

*n.a.: not available.

According to published breakpoints, the percentage of resistant isolates to Flu was 3% for Cg and 0% for Ca, Cp, and Ct; to Itra 70% for Cg, 7% for Ct, 1% for Ca and 0% for Cp; to 5FC 18% to Ct, 2% for Ca and 0% for Cg and Cp. Although no breakpoints are available for Ravu and Vori, a few resistant strains ($\geq$1 mg/L) were isolated among nonalbicans species [Ravu: Cg(5), *C. guilliermondii* (2), *C. krusei* (1), Ct(3); Vori: Cg(2), *C. guilliermondii* (2)].
Conclusions: In 19 European medical centers, *C. albicans* represented 2/3 of all clinically significant *Candida* species between 1999 and 2001. Resistance to azoles was similar for *Candida* species isolated from BSI compared with those isolated from other sites. In particular, Ravu and Vori demonstrated an excellent in vitro activity against all *Candida* species tested.

P714 Prevalence and resistance rates of *Enterobacter* spp. in nosocomial infections, Euro-SENTRY Antimicrobial Surveillance program, 1997–2001

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Objective: *Enterobacter* spp. are reported as emergent multiresistant nosocomial pathogens in several countries. The SENTRY program monitors antimicrobial resistance of nosocomial and community-acquired pathogens via an international network of hospitals. The prevalence and antimicrobial resistance rates in *Enterobacter* spp. isolates from European SENTRY hospitals were analyzed for the years 1997–2001.
Material and methods: A total of 10–27 hospitals in 17 European countries collected 32.921 isolates from bloodstream infections (BSI), lower respiratory tract infection (LRTI), wound and urinary tract infection (UTI). Antimicrobial susceptibility to 28 antimicrobial agents was tested by a broth microdilution NCCLS method in two central monitoring centers.
Results: *Enterobacter* spp. ($n = 1587$) were recovered, with *E. cloacae* (EC) and *E. aerogenes* (EA) as predominant species (69 and 24%, respectively), and 9 other species (7%). The proportion of infections caused by *Enterobacter* was 4% of BSI per year, 5.5–9.1% of LRTI per year; 40% were from patients admitted to intensive care units (ICU). EA isolates were significantly more resistant than EC isolates to cephalosporin, penicillins, aminoglycosides, and fluoroquinolones. Resistance rates were higher in isolates from ICU patients than non-ICU in both species. In 2001, the proportion of non-susceptible isolates were for EA/EC, respectively: ceftazidime 31.2/15.3% (52.5/34.6% in ICU), cefepime 0/2.2% (0/1.2% in ICU), piperacillin-tazobactam 25/15.3% (42.5/33.3% in ICU), ciprofloxacin 5.1/5.1% (37.5/16% in ICU), amikacin 0/2.9% (2.5/1.2% in ICU), and gentamicin 6.2/6.6% (12.5/12.3% in ICU). Only four nonsusceptible carbapenem strains were recovered since 1999 (three EA and one EC). Multiresistant EA were mainly recovered from France (23–86% of EA per year), Belgium (20–66% of EA per year) and Greece (60–100% of EA per year).
Conclusions: The data confirm the high prevalence of *Enterobacter* species as a nosocomial pathogen. Higher resistance rates were observed in *E. aerogenes* and in ICU patients. Susceptibility to the most potent antimicrobials (carbapenems, cefepime and amikacin) remained stable at >90% of isolates.

P715 Carbapenem-resistance among hospitalized patients in the Asia-Pacific Region (APAC): report from the SENTRY Antimicrobial Surveillance Program, 1998–2001

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Objective: Resistance to carbapenems in *Pseudomonas aeruginosa* (PAER) and Acinetobacter (ACIN) species is being described with increasing frequency in many parts of the world. Because carbapenems are now commonly used in hospital practice in the Asia-Pacific region (APAC), we wished to determine whether carbapenem-resistance was emerging in PAER and ACIN isolates from hospitalized patients.
Methods: As part of the SENTRY APAC we examined PAER and ACIN isolates from infected patients in seven countries (16 laboratory centers) since 1998. Isolates came from blood, LRTI, skin/skin structure, urine, and intensive care specimens in hospitalized patients, and were tested by broth microdilution against a wide range of antimicrobials including imipenem (IMI) and meropenem (MER), over the concentration range 0.06–8 μg/mL. Isolates were considered resistant to either of these carbapenems if their MIC was >8 μg/mL.
Results: A total of 1830 PAER and 588 ACIN isolates were collected over the 4-year period. Rates of MER resistance by country and year are shown in the Table 1. Examination of the antibiograms of the ACIN from the two countries where resistance was seen most frequently (Singapore and Taiwan), indicate that clonal spread may have accounted for up to one half of all the resistance seen. Over 90% of MER-resistant ACIN had a possible metallo-beta-lactamase phenotype (ceftazidime- and meropenem-resistant).

Table 1

	ACIN				PAER			
Country	1998	1999	2000	2001	1998	1999	2000	2001
Australia	0	0	0	0	3.4	2.8	5.4	4.0
Hong Kong, China	15.4	4.2	0	0	8.3	9.5	9.3	11.8
Mainland, China	0	8.3	–	–	11.9	15.4	–	–
Japan	6.3	7.7	0	7.1	8.3	8.7	8.7	26.2
Philippines	0	0	0	0	10.0	10.5	6.9	6.9
Singapore	10.5	20.0	33.3	33.3	0	16.7	3.3	9.1
South Africa	0	0	8.8	5.3	14.8	0	13.2	4.4
Taiwan	0	14.3	5.6	21.2	0	0	3.3	5.7

Conclusions: Carbapenem-resistance in ACIN was common in only two countries in the APAC, and appears to vary significantly from year-to-year, perhaps related to changes in circulating clones over time. MER resistance in PAER appears to be stable in the APAC region except for a recent (2001) rapid rise in Japan.

P716 Antimicrobial susceptibility and epidemiology of Australian and South African *Neisseria meningitidis* from the SENTRY Asia-Pacific Surveillance Program, 2001

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Objectives: The emergence of meningococcal strains with reduced susceptibility to penicillin has been reported in several countries during the past two decades. As part of the SENTRY Asia-Pacific surveillance program clinical isolates of *Neisseria meningitidis* (NMEN) collected during 2001 were examined for prevalence of antibiotic resistance and epidemiological characteristics.
Methods: Susceptibility of isolates to penicillin, ceftriaxone, ciprofloxacin, rifampicin and trimethoprim-sulphamethoxazole (SXT) were determined using Etest strips on Mueller-Hinton agar supplemented with 5% sheep blood. All isolates were further characterized using the automated RiboPrinter® system (DuPont Qualicon) with *Eco*RI restriction enzyme.
Results: A total of 43 isolates were tested. 95% of isolates were from blood or CSF. One isolate from sputum was penicillin non-susceptible (MIC, 0.25 mg/L). Over 70% were susceptible to SXT at $\leq$0.5 mg/L. One strain (CSF, South Africa) had SXT MIC of 3 mg/L. All strains were highly susceptible to ciprofloxacin, ceftriaxone and rifampicin. No beta-lactamase production was detected. Over 80% of isolates from Australia were serogroup B (see Table 1). In South Africa, however, serogroups A, B, W135 and Y were found in similar numbers. Over 25 different ribogroups have been detected to date, with more than nine small clusters. A comparison with other fingerprinting methods is ongoing.

Table 1

	Serogroup					
Country	A	B	C	W135	Y	Total
Australia		16	2		1	19
South Africa	7	7		7	3	24

Conclusions: Reduced susceptibility to penicillin was uncommon in Australia and South Africa. Automated ribotyping enables riboprint profiles to be easily and reliably performed, which allows for timely comparison of isolates from different geographic regions. Best discrimination was observed, however, when a combination of typing methods were used.

P717 Evolution of an integron carrying blaVIM-2 in Central Europe: report from the SENTRY Antimicrobial Surveillance Program

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Objectives: To analyze the genetic nature and context of the determinant of imipenem resistance in a blood stram isolate of *Pseudomonas aeruginosa* isolated in Poland as part of the SENTRY Antimicrobial Surveillance Program.
Methods: The determinant of imipenem resistance was isolated from a gene bank of *P. aeruginosa* strain 81–11963A prepared in the cloning vector pK18, by plating out on media containing ceftazidime plus the serine lactase inhibitor BRL42715. Sequencing was carried out on both strands by the dideoxy-chain termination method with a Perkin-Elmer Biosystems 377 DNA sequencer and sequence analysis was performed using DNASTAR software.
Results: The metallo-beta-lactamase blaVIM2 was found on a 5 kb insert of chromosomal DNA containing the transposon gene tnpr and a Class1 integron harboring two gene cassettes (*aacA4* and *blaVIM2*). The integron shows high identity to the blaVIM2 containing integron In58 previously reported in France. However, the blaVIM2 integron is novel in possessing a gene cassette that has a 59-bp element that is only 19 bp long and is probably fused to the downstream *aacA4* gene cassette.
Conclusions: The deleted 59be is not expected to be functional which would indicate that the *blaVIM2* gene is no longer a component of a functional blaVIM2 cassette but rather is part of a double gene cassette that also carries the aminoglycoside resistance gene *aacA4*. This is the first report of a mobile metallo-beta-lactamase in central Europe and illustrates the continuing dissemination of these genes throughout the continent. The enzymes encoded by these mobile genes can hydrolyze almost all clinically useful beta-lactams, irrespective of class, and the fact they are resistant to the effects of serine beta-lactamase inhibitors makes such reports particularly alarming for selection of clinical treatments.

P718 Epidemiology of the new metallo-beta-lactamase gene, blaSPM, in South America: report from the SENTRY Antimicrobial Surveillance Program

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Objective: Recently, a new type of metallo-beta-lactamase genes, blaSPM, was described from Sao Paolo, Brazil; however, very little is known of the epidemiology of blaSPM. Therefore, as part of the SENTRY Antimicrobial Surveillance Program, we genetically analyzed *Acinetobacter* spp. or *Pseudomonas aeruginosa* from Argentina and two centers in Brazil for the presence of the *blaSPM* gene.
Methods: Phenotypic screening was carried out by the Etest MBL strip (AB BIODISK, Solna, Sweden). Biochemical analysis utilized imipenem hydrolysis in the presence of EDTA (20 mM) or BRL42715 (1 mM). Amplification of the SPM gene was performed by PCR using AB-gene Expand Hi-fidelity master mix PFU/proof reading TAQ polymerase. Sequencing of PCR amplicons was undertaken by the dideoxy-chain termination method. Sequence analysis was performed using the Lasergene DNASTAR software. Alignments and phylogenetic analysis was obtained using Clustal W and PAM 250 matrix.
Results: Isolates meeting the criteria and screened were: 12 from Argentina and 19 from Brazil. Of the 19 isolates from Brazil, 18 showed imipenem hydrolysis inhibited by EDTA. PCR screening showed that 11 isolates carry the blaSPM gene; 10 from same center as the index blaSPM gene and one from a new center in Brazilia. Seven of the Brazilian are likely to carry novel, as yet unidentified, metallo-beta-lactamases. Sequencing analysis has revealed the blaSPM PCR products to be identical to that of blaSPM-1. Biochemical analysis on the isolates from Argentina revealed that most possess weak imipenem hydrolytic activity and none gave positive PCR products for blaSPM.

Conclusions: The data from this study indicates that the blaSPM gene has already been found in other Brazilian cities other than Sao Paolo. This data also indicates that bacteria strains from this region carry metallo-beta-lactamase genes other than the blaSPM as judged by their phenotypic profile. The data from the Argentinean centers suggest that the *blaSPM* gene has not spread to that country. The findings of this study highlight the importance of large-scale surveillance, molecular-level studies.

P719 Antimicrobial susceptibility and serotypes of *Streptococcus agalactiae* clinical isolates from a Lisbon hospital

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Objectives: *S. agalactiae* is a major pathogen in neonates but can also cause several infections in adults. This study determined the prevalence, antimicrobial susceptibility and serotypes of clinical isolates of *S. agalactiae* in the largest Lisbon hospital over a 3-year period.
Methods: From December 1999 to November 2002 all *S. agalactiae* recovered in the Laboratory of Bacteriology of Hospital de Santa Maria were studied. Susceptibility testing was performed by disk diffusion (according to NCCLS) and *E*-test. Antimicrobials tested included penicillin, cefotaxime, erythromycin, clindamycin, tetracycline, chloramphenicol, ofloxacin, and vancomycin. Serotyping was done by slide agglutination using antisera (Hemolytic streptococcus Typing antisera for Group B, Denka Seiken, Japan) according to the instructions of the manufacturer.
Results: A total of 252 *S. agalactiae* were isolated from clinical samples. The origin of isolates was: blood (37), urine (54), sterile fluids (4), vaginal swabs (108), and other products (49). Invasive isolates were detected from (17) neonatal infections and (24) adult infections. Resistance to antimicrobial agents was detected against erythromycin (10.7%), clindamycin (9.9%), and tetracycline (75.4%). All isolates were susceptible to penicillin (MIC50 = 0.064 mg/L; MIC90 = 0.094 mg/L), cefotaxime, chloramphenicol, ofloxacin, and vancomycin. Erythromycin resistant phenotypes were MLSB constitutive (70.4%), MLSB inducible (22.2%), and M phenotype (7.4%). Serotype distribution was as follows: Ia (17.9%), Ib (3.6%), II (16.6%), III (24.2%), IV (2.0%), V (23.4%), VII (1.2%), and VIII (0.4%). A total of 10.7% were non-typable.
Conclusion: Penicillin was active against all strains but macrolide resistance was detected in 10.7% isolates. Overall, the most common serotypes were III, V, Ia, and II. Within the 41 invasive isolates the serotypes ranked as follows: III (15), Ia (10), V (7), II (3), IV (3), Ib (2), and VII (1). *S. agalactiae* infections are caused by a heterogeneous group of microorganisms, according to susceptibility pattern and serotype.

P720 Resistance and serotypes of *Streptococcus pneumoniae* invasive isolates from Portugal

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Objectives: Determine the antimicrobial susceptibility and serotype of invasive isolates of *Streptococcus pneumoniae* recovered in Portugal during a 4-year period.
Methods: *S. pneumoniae* recovered from normally sterile sites during the years of 1999–2002 were sent to the Microbiology Laboratory of Faculdade de Medicina de Lisboa for study. Susceptibility testing was performed by disk diffusion (according to NCCLS guidelines) and *E*-test. Antimicrobials tested included penicillin, amoxacillin/clavulanic acid (XL), cefuroxime (XM), cefotaxime (CT), erythromycin (EM), clindamycin (CM), tetracycline (TC), chloramphenicol (C), trimethoprim-sulfamethoxazole (SXT), levofloxacin (LE), moxifloxacin (MX), gatifloxacin (GA), linezolid (LZ), quinupristin/dalfopristin (RP), and vancomycin. Serotyping was done by the Quellung reaction using sera from the Statens Serum Institut (Copenhagen, Denmark).
Results: The origin of the 407 isolates tested was: blood (384), CSF (13), other sterile fluids (10). Resistance to most antimicrobial agents was detected: XL 4.9%, XM 14.5%, CT 1.5%, EM 8.4%, CM 6.1%, TC 5.6%, C 2.4%, SXT 19.5%, LE 0.2%. Four isolates presented low-level resistance to quinupristin/dalfopristin (MIC = 1.5 mg/L). All isolates were susceptible to moxifloxacin, gatifloxacin, linezolid, and vancomycin. Penicillin non-susceptibility was 19.9% (MIC50 = 0.023 mg/L; MIC90 = 1 mg/L). Low-level resistance was observed in 63 isolates whereas high-level resistance was restricted to 18 isolates. Multiresistance was detected in 7% of the isolates. Six serotypes accounted for half of all isolates: 1 (11.8%), 14 (11.6%), 3 (10.8%), 4 (6.7%), 8 (6.7%), and 9V (4.9%). The rank order and serotypes accounting for half of the isolates varied with the age group considered.
Conclusion: Penicillin non-susceptibility was mostly (77%) associated with serotypes 9V, 14, and 23F found in internationally disseminated clones. As expected, multiresistance is also found mainly among these serotypes as well as 19A and 6B. Despite 20% non-susceptibility to penicillin, most strains remain susceptible to amoxacillin/clavulanic acid (95.6%), third generation cephalosporins (98.5%) as well as to new antimicrobials soon to be made available or recently introduced in Portugal – levofloxacin, moxifloxacin, gatifloxacin, quinupristin/dalfopristin and linezolid. Coverage of the 23-valent polysaccharide vaccine is almost constant among all age groups and is 90% among individuals older than 60 years of age.

P721 Antibiotic susceptibility of *Edwardsiella* isolates from Antarctic penguins

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Objective: The spread of antibiotic resistance is generally thought to be a result of the use of antibiotics, but sporadically it has been suggested that resistance can be common even in environments apparently devoid of antibiotic exposure. Here we look at Enterobacteriaceae from such an environment.
Methods: Fecal bacteria from cloacal swabs of gentoo penguins (*Pygoscelis papua*) living on the Antarctic Peninsula were isolated from MacConkey plates. Enterobacteriaceae were identified by biochemical tests, and in 17 cases, 16*S* rDNA sequencing. MICs to 17 antibiotics were determined by agar dilution.
Results: From 14 swabs of 49, enterobacteria were found; in all 42 isolates. Of these, 38 were *Edwardsiella tarda*, or closely related species (100% sequence similarity for 10 isolates, 99–97.6% for 5 isolates sequenced). The other isolates were one *E. coli*, and three unknown species. All isolates were susceptible to all antibiotics tested. Compared with median MIC values of susceptible Enterobacteriaceae in human fecal flora, the *E. tarda* MICs were three to five dilution steps lower for ampicillin, amoxiclav, piperacillin-tazobactam, cephalothin, cefuroxime, ceftazidime, trimethoprim, and chloramphenicol. Nalidixic acid, tetracycline, gentamicin, streptomycin, and sulphamethoxazole only differed 0–2 steps. The extreme susceptibility caused technical problems: antibiotic carryover from the highest dilutions in normal dilution series (e.g. 0.06–128 mg/L) inhibited the growth of many strains on the control plates of the next series. Therefore, low-range series, ending at 8 or 4 mg/L had to be used.
Conclusion: In line with our previous results on Enterobacteriaceae from vegetables and wild Finnish moose, deer and vole, no acquired resistance was found in the fecal microbiota of Antarctic penguins. Resistance is thus acquired and spread mainly due to antibiotic use; microbiota in pristine environments are naturally susceptible.

P722 Molecular epidemiology of penicillin-resistant *Streptococcus pneumoniae* in Turkey

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Objective: To investigate the clonal relationship among penicillin-resistant and penicillin-susceptible *S. pneumoniae* strains recovered from different medical centers in Turkey.
Methods: A total of 110 *S. pneumoniae* (10 high level (PenR), 80 low-level (PenI) penicillin-resistant and 20 penicillin-susceptible) isolates selected randomly from seven centers, were studied. Penicillin (Pen) and erythromycin (E). MICs were analyzed by *E*-test (AB Biodisk). Cefotaxime MICs were determined by microdilution. Susceptibility patterns for other agents, namely clindamycin (CC), tetracycline (Te), chloramphenicol (C), trimetophrim sulfamethoxazole (SXT), ciprofloxacin and levofloxacin were studied by disk diffusion. Capsular serotypes were determined by coagglutination and quellung reactions. Clonal relationship among the isolates and similarities of the patterns with 16 penicillin-resistant clones defined by the Pneumococcal

Molecular Epidemiology Network (PMEN) were investigated by BOX-PCR using BOX-A1R primer.
Results: Most of the PenR isolates were also resistant to other antibacterial agents but not to levofloxacin. Quinolone resistance was not observed. Serotypes 23, 14 and 9V were the most frequent serogroups among PenR isolates. Eight, 57 and 18 unique molecular patterns were identified among high-level PenR, low-level PenR and PenS strains, respectively. Although, six and seven Ankara isolates showed similar BOX-PCR patterns with Spanish 23F and 6B clones, respectively, most of the Turkish isolates were different from the PMEN clones.
Conclusions: According to BOX-PCR patterns, most of the penicillin nonsusceptible *S. pneumoniae* isolated in Turkey, were diverse clonally.

P723 Epidemiological study of vancomycin-resistant *Enterococcus* isolated from a single medical university hospital in Japan

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Objectives: Since 1998 more than 130 reports have described the isolation of high-level vancomycin-resistant enterococci (VRE) in Japan. Here, we report on our clinical isolates of VRE and an epidemiological study carried out using chemical and genetic techniques.
Methods: VRE isolates were screened for high resistance to vancomycin (VCM) with a cutoff value of 6 μg/mL and VCM-resistant gene was confirmed by polymerase chain reaction (PCR). The epidemiological studies used pulse-field gel electrophoresis (PFGE) and plasmid analysis.
Results: Seven strains of VRE were isolated from seven different patients on three wards during early 3-month period, and five strains of VRE were isolated from five different patients on three wards during late 3-month period. All of the isolates possessed vanA on their plasmid, and the isolates of early group were divided into two similar groups. Furthermore, three different patterns were defined by PFGE. Although all of the asymptomatic carriers were hospitalized for more than 3 months, we were able to prevent an outbreak of VRE in our hospital by using our guidelines for infection control, which are stricter than those of methicillin-resistant *Staphylococcus aureus*.
Conclusions: From the results of this epidemiological study, we propose that there was a possibility of contamination in this hospital, and that three of the early seven isolates may have acquired vanA independently. In this study we demonstrated that the infection control, according to appropriate prevention guidelines, as well as regular surveillance for VRE, are essential for designing interventions to prevent the further spread of VRE.

P724 Epidemiology and antibiotic susceptibility of common bacteria causing skin and soft tissue infections in the USA, Canada, and Europe (TSN Database 2000–2002): a guide to empiric therapy

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Objectives: In this study we report current incidence and susceptibility rates of antimicrobials for key pathogens isolated from skin and soft tissue infections (SSTI) as reported to physicians by clinical microbiology laboratories in Europe (EU), Canada (Cn) and the USA.
Methods: We analyzed data (January 2000–October 2002) from The Surveillance Network® (TSN) Databases in France (Fr), Italy (It), Germany (Gy), Spain (Sp), Cn and the USA to determine susceptibilities from routine test results as reported to physicians. Only inpatient data from isolates taken from SSTI ($n = 276{,}739$) were included in the analysis. Contemporary NCCLS breakpoints were used, except for Fr (CA-SFM).
Results: Gram-positive organisms (*S. aureus* [SA]), *Streptococcus* spp. and *Enterococcus* spp. [Esp] and Enterobacteriaceae (notably *E. coli* [EC]) were the most common isolates among SSTI. Methicillin-resistant *S. aureus* (MRSA) rates were USA 45.7%, It 41.0%, Fr 34.6%, Sp 34.2%, Gy 12.0% and Cn 18.1%. Greater than >99.0% of methicillin-susceptible *S. aureus* (MSSA) tested susceptible (S) to ceftriaxone (CTX) and >94.9% to trimethoprim-sulfamethoxazole. A total of 83.6% (Fr) to 92.7% (Gy) of MSSA were ciprofloxacin-S; 71.3% (USA) to 87.9% (Sp) were erythromycin (ERY)-S; 81.1% (It) to 99.1% (Fr) were gentamicin-S. 100% of *S. agalactiae* and *S. pyogenes* were S to penicillin, CTX and cefotaxime (CFO). ERY-R was 11.7% (Sp) to 22.2% (USA) for *S. agalactiae*, 10.8% (Gy) to 25.4% (It) for *S. pyogenes* and 14.1% (Gy) to 28.0% (Fr) for viridans group streptococci. Vancomycin-R Esp were uncommon outside the USA (16.0%) and It (4.1%). EC from all countries were 100% imipenem-S, >98.6% to amikacin (AK), >90.4% to CTX and CFO. Putative extended-spectrum β-lactamase expression in EC remained rare, comprising 3–7% of isolates in USA, It, Sp, and Cn and <2% in Fr and Gy. For *P. aeruginosa* piperacillin-tazobactam, AK, IMI and ceftazidime were the most active compounds tested irrespective of region.
Conclusions: Among SSTI, rates of MRSA, vary significantly between countries. Resistance to orally available compounds was common in all species. Surveillance data should be considered when selecting empiric therapy for treating SSTI.
Conclusions: Among SSTI, rates of MRSA, vary significantly between countries. Resistance to orally available compounds was common in all species. Surveillance data should be considered when selecting empiric therapy for treating SSTI.

P725 Pitfalls in prompt eradication of nasal MRSA (methicillin-resistant *Staphylococcus aureus*) carrier status in health care workers

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Objectives: The aim of the study was to investigate the effectiveness of eradication treatment in nasally colonized health care workers (HCWs) and to highlight reasons for eradication failure.
Methods: Two tertiary care hospitals (1600 beds) in South-west Germany perform an active screening of HCWs, who had close contact to MRSA patients. MRSA carrying HCWs are treated with local antibiotics (mupirocin, tyrothricin) and antiseptic soap for 5 days and are advised to clean and disinfect bath room, hygiene articles and bed linen. Screening for successful eradication is performed for up to 3 months. When eradication failed, additional screens are performed, including the animate and inanimate home environment. Infection control charts of patients and HCWs for a 7-year period (1995–2001) were reviewed.
Results: From 1995 to 2001, a total of 592 patients were found to be MRSA-carriers; 87 HCWs harbored MRSA in their noses soon after contact to these patients. 73 of 87 (84%) of HCWs responded with prompt and definite eradication of their carrier status. Pitfalls of this eradication strategy were observed in 14 HCWs; all but three were tested MRSA negative shortly after eradication treatment, but were recolonized 10–30 days thereafter. In two of three cases where swabs did not turn negative, initial eradication had failed because of inadequate eradication treatment; when repeated and intensified, they turned definitely negative. In 11 of the recurrent cases, household contacts (HC) were screened for nasal carriage; in 8 of these cases, environmental samples were taken from the home environment. HC of 8 out of 11 carriers also proved to be colonized. In 7 out of 8 screened environments MRSA was detected although HCWs had cleaned bed and bath rooms. Two homes were shown to be heavily contaminated, suggesting long-term carriage by the household members. When eradication therapy was given to HC and surfaces were cleaned and disinfected, prompt and definite eradication was achieved in most cases within a few weeks; however, in the cases of heavily contaminated homes, definite eradication success was achieved only by architectural renovation/sanitation and took up to 1 year to achieve.
Conclusions: The data support the hypothesis, that attempts to eradicate long-term carriers (patients or staff) should not be restricted on antibiotic or antiseptic treatment, but must also include cleaning and disinfection of household contamination.

P726 Diversity and spread of multiresistant *Acinetobacter baumannii* strains in Europe

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Objectives: To explore the diversity and spread of multiresistant *Acinetobacter baumannii* strains in Europe
Methods: Fifty geographically representative isolates were investigated that had been allocated to ribogroups 1, 2 and 3 in a previous study. In that study, 400 isolates were collected from 23 European hospitals. Amongst these, 145 sitofloxacin-resistant Acinetobacter isolates were recognized, 92 of which

were allocated by automated ribotyping to three major ribogroups 1, 2 and 3 with 17, 52 and 23 isolates, respectively. Species identification of the 50 selected strains of the present study was performed by amplified ribosomal DNA restriction analysis (ARDRA), and by AFLP fingerprinting cq cluster analysis with fingerprints of reference strains of all described (genomic) species using a clustering level of 50% to delineate species. The diversity at strain level was investigated by high resolution fingerprinting using pulsed field gel electrophoresis (PFGE) and AFLP using a 90% level for strain delineation.
Results: All isolates were multiresistant and were identified as *A. baumannii*, confirming the predominance of this species in clinical settings. Combined AFLP and PFGE led to the distinction of 23 genotypes within the, with PFGE being the most discriminatory method. Cluster analysis of PFGE and AFLP profiles showed a good agreement with ribogroup categorization, except for two PGE types. Most genotypes corresponded to a single hospital, indicating the local spread of a single strain, whereas three genotypes were found in different geographic locations. The AFLP fingerprints of these strains were distinct from the previously described identified NW European clones 1 and 2. Isolates of these three widespread genotypes showed different antibiotic susceptibility profiles in different hospitals.
Conclusions: Grouping by ribotyping seems a useful method for rough typing of strains, whereas AFLP and/or PFGE provide reveal additional genotypes and AFLP and/or ARDRA are more useful for strain identification. The combined use of the methods revealed the existence of several widespread multiresistant strains.

P727 Urinary tract infections in Northern Greece: bacterial etiology and susceptibility. A retrospective study of clinical isolates

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Objectives: The aim of this study was to interpret the results of urine cultures which were sent in the laboratory of the University Hospital AHEPA from 1998 until 2002 both from hospitalized and outpatients – population of Northern Greece – so as to identify major bacterial isolates which are responsible for urinary tract infections and to test their sensitivity to antibiotics.
Methods: 24259 urine samples were cultivated during the above mentioned period. Samples were inoculated in ordinary used media: blood and McConkey agar. The identification of bacterial isolates and antibiotic susceptibilities were performed by the analyzer VITEK 60 of BIOMERIEUX.
Results: 5445 out of 24259 urine samples had positive results. More specifically, bacteria in hospitalized patients – 4529 in total – were isolated as follows: *E. coli* in 2291 patients (56%), *Pseudomonas aeruginosa* in 445 (10%), *Enterococcus* spp. in 342 (7.5%), *Klebsiella* pn. in 309 (7%), *Proteus* spp. in 270 (6%). Bacteria-916 in total isolated from outpatients appeared as follows: *E. coli* in 636 patients (69%), *Proteus* in 77 (8%), *Enterococcus* in 47 (5%), *Klebsiella* pn. in 45 (5%), *P. aeruginosa* in 34(4%). The antibiotic sensitivity of *E. coli* was: Amoxicillin/Clavulanic 80%, Cefuroxime 88%, Ciprofloxacin 92%, Norfloxacin 92%. Trimethoprime/Cotrimoxazole 78%, Ticarcillin/Clavulanic 85%, Tobramycin 98%. The antibiotic sensitivity of Proteus was: Amoxicillin/Clavulanic 83%, Cefuroxime 83%, Ciprofloxacin 89%, Norfloxacin 91%, Trimethoprime/Cotrimoxazole 70%, Ticarcillin/Clavulanic 98%, Tobramycin 92%. The antibiotic sensitivity of *P. aeruginosa* was: Amikacin 70%, Cefepime 64%, Ceftazidime 81%, Ciprofloxacin 64%, Imipenem 76%, Piperacillin/Tazobactam 86%, Ticarcillin/Clavulanic 63%, Tobramycin 71%.
Conclusions: Our study indicates that (1) *E. coli* is the most common cause of urinary tract infection mostly sensitive to tobramycin, ciprofloxacin and norfloxacin. The observed decrease in susceptibility to cotrimoxazole is worrying. (2) *P. aeruginosa* was mostly sensitive to piperacillin/tazobactam, ceftazidime, tobramycin. A higher resistance of *P. aeruginosa* to imipenem was observed in nosocomial infections. (3) *Proteus* was mostly sensitive to Ticarcillin/Clavulanic, tobramycin and norfloxacin. (5) The bacteria isolated from hospitalized patients suffering from urinary tract infection appeared higher resistance in the used antibiotics than bacteria isolated from outpatients.

P728 Comparison of several criteria for rejecting multiple isolations on *Pseudomonas aeruginosa* antimicrobial sensitivity estimation

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Objectives: To compare several criteria for rejecting duplicate isolations and to know its effects on the estimation of antimicrobial sensitivity of *Pseudomonas aeruginosa* during a 2-year period (2000–2001).
Methods: We applied the following criteria:
1. Time elimination criterion: We have calculated data on sensitivity by rejecting multiple isolates from the same patient within an interval of 0 (All), 7, 15, 21 and 30 days by means of ViResiST program (vvv.viresist.org).
2. Antibiotic sensitivity criterion: By means of Wider system (Soria Melguizo, Spain), we have calculated sensitivity data by rejecting multiple isolates from the same patient that show the same antibiogram, regardless of time criterion.
Results: The average isolation rate (AIR) (number of isolates tested/number of patients tested) was 1.6, the frequency of single isolation (FSI) (number of patients with single isolations/number of patients tested) was 0.7. The estimated sensitivity percentage appears in Table 1.

Table 1

	Time elimination criterion					
Sensitivity to	0 days (all isolates)	7 days	15 days	21 days	30 days	Antibiotic sensitive criterion
Ceftazidime	91.47	92.27	92.99	92.74	93.23	90.97
Imipenem	88.42	89.77	90.65	91.57	92.02	86.46
Ciprofloxacin	80.87	80.58	81.07	82.20	83.09	78.94
Amikacin	92.92	92.27	95.09	95.55	95.65	88.90

Conclusions: Data of longest intervals suggest that it is likely that there are more duplicate isolates when patients are infected by multiresistant microorganisms. The use of antibiotic sensitivity criteria detects strains that became resistant during the course of the treatment and there fore the results show lower percentages of sensitivity. Antibiotic sensitivity criterion is the one which reflects the real situation best, but it may be influenced by methodological mistakes, for this reason sensitivity of strains that show mutations during treatment should be confirmed.

P729 Vancomycin resistance of *Staphylococcus aureus* isolates from co-colonized patients with vancomycin-resistant enterococci and *Staphylococcus aureus*

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Objectives: Transfer of vancomycin resistance determinants from vancomycin-resistance enterococci to *Staphylococcus aureus* has been a serious concern. It has been shown that such a transfer was possible in the laboratory. More recently, two clinical isolates of true vancomycin-resistant *S. aureus* having vanA were reported. We examined the vancomycin resistance of *S. aureus* isolates from cocolonized patients with *S. aureus* and VRE including rectal or perianal regions where two organisms might be encountered.
Methods: During a year (2001), swabs of the nare, axilla, perineum and rectum were obtained weekly to isolate *S. aureus* among the patients known to be colonized with VRE from clinical specimens or rectal surveillance cultures. MICs of vancomycin and oxacillin of *S. aureus* isolates were determined by NCCLS broth microdilution test.
Results: A total of 228 swabs were conducted from 19 patients who were colonized VRE. Ten of 19 patients had *E. faecium* or *E. faecalis* (vancomycin MIC 256 μg/mL and 9 patients had *E. gallinarum*, *E. casseliflavus* or *E. avium* (vancomycin MIC 4–16 μg/mL). 60 isolates of *S. aureus* were collected and 58 strains (96.7%) were MRSA (24, 6, 13, 15 isolates from nare, axilla, perineum

and rectum). Vancomycin MIC of MRSA isolates was as follows: 2, 49, 6 and 1 isolates for MIC of 0.5, 1, 2 and 4 μg/mL.
Coclusions: Despite high percentage of MRSA colonization, no VRSA strains were recovered among cocolonized patients with *S. aureus* and VRE.

P730 Prevalence of extended-spectrum beta-lactamases in a district general hospital

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Introduction: Extended-spectrum beta-lactams are commonly included in empirical antibiotic regimens for the treatment of Gram-negative sepsis. However, the emergence of extended-spectrum beta-lactamase (ESBL)-producing bacteria pose a serious threat to the continued use of this family of antibiotics.
Objectives: Following the isolation of Enterobacteriaceae producing ESBLs from patients in the Haematology unit at York District Hospital during 2000–2001, a study was instigated to look at the incidence of ESBL producing isolates from blood culture taken from the general hospital population.
Material and methods: 234 selected clinical isolates of Gram-negative bacilli obtained from blood culture taken from inpatients at York District Hospital, York, UK between 1997 and 2001 were studied. The selected isolates were all resistant to at least one of the following extended-spectrum beta-lactams; ceftriaxone, ceftazidime, cefotaxime. The isolates were identified using the API 20E system. All isolates were tested for ESBL production by the double disc diffusion test and the MAST DD Test. The Minimum Inhibitory Concentration of ceftriaxone, ceftazidime, cefotaxime, cefepime, cefoxitin and imipenem were determined for all the isolates using an agar dilution method. Specific polymerase chain reaction (PCR) was used to screen for the presence of blaSHV, blaTEM and blaCTX-M in the ESBL-producing isolates. Pulsed Field Gel Electrophoresis (PFGE) was used to determine the clonality of the isolates.
Results: ESBL production was detected in 83 isolates, 62 *Escherichia coli*, 10 *Klebsiella pneumoniae*, 5 *Klebsiella oxytoca*, 2 *Proteus mirabilis* and 4 *Enterobacter cloacae*. PFGE revealed the presence of multiple clones. Molecular characterization of the ESBLs showed that the isolates produce SHV-5, SHV-12, TEM-52 and CTX-M ESBL.
Conclusion: To our knowledge, this is the first report describing the presence of organisms expressing ESBL in a District General Hospital in the UK. The data presented here highlight the global emergence of these clinically important resistance determinants and the importance of screening for their presence not only in tertiary hospitals but in district hospitals as well.

P731 Teicoplanin-resistant *Staphylococcus aureus* from diabetic food ulcer

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Objectives: The emergence of glycopeptide resistance in *Staphylococcus aureus* (MRSA) causes major concern as therapy often relies on these compounds. We report a case cluster of infections with Teicoplanin-resistant MRSA.
Methods: Susceptibility tests were performed using *E*-test strips. For the detection of resistance genes, PCR and chip-based hybridization assays were used. PCR assays for the coa gene were performed for preliminary typing based on polymorphisms of that gene.
Results: A MRSA was isolated from a diabetic food ulcer of a patient from an outpatient department in which that drug is administered routinely. The MIC for Teicoplanin was 16–24/mL but the MIC for Vancomycin was 2 μg/mL. VanA and vanB were not detectable. The isolate contained *mecA*, *blaZ*, *apha3*, *norA*, and *sat* genes. It belonged to the Berlin epidemic strain. In PCR assays for the coa gene, the resistant isolate showed the same pattern as a Teicoplanin-susceptible MRSA isolate from the same patient which was sampled 5 weeks earlier. Possible transmissions to other patients are investigated after Teicoplanin-resistant MRSA with identical coa patterns were isolated in three additional cases.
Conclusions: The present set of data might indicate a mutational resistance which developed under therapy and a subsequent spread to other patients.

P732 Characterization of macrolide and streptogramin-resistant genes in enterococci from poultry and human samples from Portugal

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Objectives: To characterize some of the genes responsible for resistance to streptogramines and macrolides in enterococci with decreased susceptibility to quinupristin-dalfopristin (QD) isolated from poultry samples (PS) and faeces of healthy volunteers (FH).
Methods: 73 PS and 46 FH were collected in Porto between 1999 and 2001. Susceptibility to erythromycin (E) and QD was performed by the agar dilution method and *E*-test, respectively. Species identification and detection of genes coding for resistance to QD or E (vatD, mefA, ermA, ermB and ermC) were done by PCR.
Results: Isolates with reduced susceptibility to QD were recovered from all samples. Enterococci resistant to E were found in 97% PS and 89% FH. Reduced susceptibility to E was verified in 3% PS and 9% FH. $N = 54$ isolates from PS (27 *E. faecium* (fc), 19 *E. gallinarum*, 8 *Enterococcus* (sp.)) and 39 from HV (fc) with decreased susceptibility to QD ($2\,mg/L < MIC > 32\,mg/L$) were included for gene characterization. vat(D) was detected only in 1 fc isolate from PS (MIC 4 mg/L). 13/22 (59%) and 31/38 (82%) E resistant isolates from HV and PS, respectively, carried erm(B). Interestingly, ermB was also found in isolates with intermediate susceptibility (MIC 1–2 mg/L): four fc FH isolates and one fc PS. No erm(B) was found in 11 isolates with MIC > 32 mg/L. Presence of erm(A), erm(C) or mef(A) was not detected in any isolate.
Conclusion: Resistant isolates to QD and E are frequently found among Portuguese PS for human consumption and HV. In agreement with other studies, the QD resistant isolates did not contain vat(D) genes suggesting that other unknown genetic mechanisms are responsible for QD resistance in enterococci. Although erm(B) gene was found in most of the E resistant isolates, it was also detected in strains with E MIC value of 1–2 mg/L suggesting that these rarely yet reported enterococci might be more widespread that it was thought. Since we did not obtain positive amplifications for *mef(A)*, *erm(A)* or *erm(C)* genes, the presence of other genetic mechanisms implicated in enterococcal E resistance cannot be ruled out and should be further studied.

P733 Genotyping of *Serratia marcescens* strains isolated over 5 years in two university hospitals

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Objectives: *Serratia marcescens* (Sm) is a pathogen difficult to treat and control. Genotyping enables the better understanding of an outbreak dynamics and endemicity. The objective of this study was to analyze Sm genotypes over long period of time at two tertiary care hospitals.
Methods: From 1996 to 2000 we collected 527 Sm isolates, 382 of them from 362 patients were selected to MIC determination by agar dilution method. Double-disk synergy test was used to detect ESBL production. RAPD was used to genotype 358 isolates, 321 from hospital I (1204 beds) and 37 from hospital II (314 beds). Fifty-six isolates selected from both hospitals were genotyped by PFGE. ESBL producers were further investigated by isoelectrofocusing and PCR.
Results: The majority of isolates were from the respiratory tract (33%), wounds (20%), urine (18%), and blood (8%). We differentiated 68 Sm genotypes by RAPD. PFGE results for selected strains were in general concordance with RAPD. Two most common strains A – 64 isolates, B – 126 comprised 53% isolates. Strain B was responsible for endemic situation in both hospitals. Five other predominat genotypes included 81 isolates. 54 genotypes were isolate specific. 35% isolates from hospital II produced ESBLs. TEM-ESBL Sm strain appeared in 1997 in hospital I but most of 67 ESBL isolates produced CTX-M enzymes and disseminated in 1999 and 2000. Endemic strains acquired ESBL enzymes but majority of ESBLs were detected in special ESBL genotypes.
Conclusions: Both methods PFGE and RAPD gave comparable results for Sm isolates obtained over 5 years. Once established Sm strains persisted for many years in two hospitals. 75% isolates belonged to seven most common genotypes. Majority of genotypes were ephemeral. CTX-M enzyme was most often produced in our Sm ESBL strains.

P734 Distribution of macrolide-lincosamide resistance amongst *S. pyogenes* isolates from different sources, during January 1998–September 2001

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Having noticed that resistance rates to erythromycin and clindamycin of *S. pyogenes* (GAS) strains, isolated from different specimen sources, differ significantly, we studied the sensitivity of GAS strains isolated during January 1998–September 2001. A total of 2744 GAS strains derived from throat (2180), ear (141), nose (101), vagina (97), pus (159) and skin lesions (66), were studied by the double-disk diffusion test, according to the NCCLS recommendations using Becton Dickinson's disks. Pharyngeal strains, during 2001, showed an increased resistance (R: 32.08%) and an intermediate one (IR: 5.75%) to erythromycin (Ery), as well as to clindamycin (Cli) with 3.98% R and 1.11% IR, compared with 21.05% Ery R, 3.95% Ery IR and inexisting resistance to Cli during 1998. Skin lesion strains, also, showed an increase in Ery resistance (1998: 10.53% R and 5.26% IR – 2001: 26.67% R and 16.66% IR). Vagina strains revealed a noticed increase of Ery resistance (1998: 14.29% Ery R and 0.00% IR – 2001: 38.90% R and 16.67% IR). None of the strains isolated from skin or vagina was resistant to clindamycin. Pus strains, in contrast, revealed a minimum resistance fluctuation (1998: 19.51% Ery R, 7.32% Ery IR and 2.44% Cli R – 2001: 18.6% Ery R, 9.30% Ery IR and 2.33% Cli R). Ear and nose isolates showed the same total Ery resistance rates, but, there was an increase in the percentage of IR strains to it, from not-existing during 1998, to 11.76% IR and 17.65% IR, respectively, during 2001. No ear isolate detected as Cli R, but, nose strains showed an increasing resistance (1998: 0% Cli R – 2001: 5.58% Cli R). The high resistance rates to erythromycin of nose strains can possibly be attributed to colonization. The increase of resistant strains totally 45% isolated from infections (pharyngo-tonsillitis, vaginitis, skin lesions) usually treated by pediatricians, possibly by macrolides, is of high importance.

P735 Beta-hemolytic streptococci from throat cultures of Greek children, during 2000–2002 – *S. pyogenes* resistance to erythromycin and clindamycin

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9812 throat cultures from children were studied using standard operating procedures, sheep blood agar 5% (OXOID), 35 °C anaerobic incubation, bacitracin 0.04 U sensitivity(OXOID), serogrouping (TransLab UK kit). Sensitivity to erythromycin (Ery) and clindamycin (Cli) was detected for all *S. pyogenes* (GAS) strains by the double disk test, using the NCCLS criteria and Becton-Dickinson's disks. A total of 2381 strains of beta-hemolytic streptococci were isolated during the 3-year period of study. The overall isolation rate and the distribution of different groups were as follow:

2000: 851 isolates with 65.33% group A, 32.31% group C and 2.35% group G.
2001: 937 isolates with 62.43% group A, 35.11% group C and 2.45% group G.
2002: 593 isolates with 71.00% group A, 27.15% group C and 1.85% group G.

The sensitivity test revealed decreasing tendency in the resistance of GAS to Ery and an increasing one to Cli, as follow:

Erythromycin resistance:
30.57% R and 4.68% IR, during 2000
25.13% R and 3.76% IR, during 2001
23.54% R and 3.32% IR, during 2002

Clindamycin resistance:
1.62% R and 0.36% IR, during 2000
3.25% R and 1.54% IR, during 2001
9.26% R and 1.66% IR, during 2002

The decline of erythromycin resistance can, possibly, be explained by the self-limitation of pediatricians in the use of macrolides these last years, as a consequence of the high resistance rates referred at late 1990s in Greece (30–35%). Clindamycin's higher resistance rate (totally 10.9%) during 2002, needs the detection of the resistance genes' distribution among GAS strains, as there is no significant change in its use.

P736 Emergence of a clone of *Streptococcus pyogenes* resistant to bacitracin during a prospective survey of acute pharyngitis in France

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Objectives: A prospective survey of acute pharyngitis was conducted from November 2000 to June 2001, in Bourgogne, a region of France, involving 20 general practitioners. The aim was to study the frequency of antimicrobial resistance of *Streptococcus pyogenes* responsible for pharyngitis.
Methods: Streptococcal pharyngitis were first diagnosed by the positivity of the Biostar Strep A Optical Immunoassay (International Microbio). It is a rapid test that detect the group A antigen. A second throat swab was cultured on Columbia blood agar plate and allowed to isolate 247 strains of *S. pyogenes* from 282 patients. Biotyping, T-typing and emm-typing were carried out with the Rapid ID Strep 32 (BioMerieux), agglutination method, and sequence analysis, respectively. Pulsed-field gel electrophoresis was performed on strains with similar markers. Susceptibility to 12 different antibiotics, including bacitracin, was tested by the disk diffusion method. The minimal inhibitory concentrations (MICs) of macrolides (erythromycin, azithromycin, and josamycin), clindamycin, and tetracyclin were determined by the agar dilution method according to the guidelines of the 'Comité de l'Antibiogramme de la Société Française de Microbiologie' (CA-SFM). The major genetic determinants of macrolides resistance (ermB, ermTR and mefA) were investigated by multiplex PCR assay.
Results: Thirty out of 247 isolates of *S. pyogenes* (12.1%) were found to be resistant to bacitracin. All these strains were also resistant to high levels of macrolides and clindamycin (MICs > 32 mg/L) associated with the ermB gene. High levels of resistance to kanamycin and streptomycin were detected in 30 and 27 strains, respectively. Most of these multiresistant strains were of biotype 1, serotype T28, and type emm28, and represent more than 40% of the emm28 strains. The pulsed-field gel electrophoresis showed an homogeneity among these strains.
Conclusions: These results confirm the spread of a strain of *S. pyogenes* biotype 1, T28, emm28, characterized by an unusual resistance to bacitracin. The spread of this clone during a 8-month period among the whole area of the survey might have been favored by the association of high levels of resistance to macrolides. Bacitracin resistant strains have already been isolated from invasive infections. Therefore, the detection of *S. pyogenes* should not be relied by the positivity of the bacitracin susceptibility test.

P737 Differences between MRSA and MSSA colonization/infection in outpatients with vascular or diabetic foot ulcers

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Objectives: To know the prevalence, epidemiology and risk factors for MRSA colonization/infection (C/I) in outpatients with ulcers as well as their differences with patients colonized/infected by MSSA.
Methods: We prospectively studied all patients with ulcers attending the Outpatient Vascular Surgery and Diabetic foot Care Clinics at our Institution. Patients were routinely cultured (nose and ulcer). A previously elaborated questionnaire was filled at each patient's visit. Demographics, previous contact with Health Care facilities, previous or actual exposure to antimicrobials, underlying disease, nature and clinical characteristics of the ulcer were recorded. We used for the comparative study *F*-Fisher and Chi-square tests for qualitative variables and *t*-Student for quantitative variables.
Results: One hundred patients were included. Sixty-four were men, mean age of 66.5 years (SD 13.8; range, 63.7–69.2), with a mean time of evolution of the ulcers of 297 days (range, 144–449). 52/100 patients had not been hospitalized during the previous year. 34% were taking antibiotics and 68% had been exposed to antimicrobials in the preceding 3 months. 72% of the patients were diabetics and 52% had arterial peripheral vascular disease. Ulcer examination revealed signs of active infection in 39%; 23% had also underlying osteitis. Six patients were colonized or infected by MRSA and 34 by MSSA; this represents an incidence of MRSA of 15% of all *S. aureus* colonized/infected patients. All MRSA patients had been in contact with Health Care facilities (hospital, ambulatory care center, etc.) prior to C/I by MRSA. MRSA patients, as compared with MSSA colonized/infected patients, were older [81 years (SD 9.8) vs. 66.2 years (SD 14.7), $P = 0.02$],

and had been exposed to antibiotics more frequently in the preceding 3 months [100% vs. 52.9%, $P = 0.03$]. MRSA strains had different resistance phenotypes and genotyping showed identity with previously isolated strains of nosocomial origin.
Conclusions: MRSA C/I in our community is rare (6%) and always found in persons previously in contact with Health Care Organizations. Old age and previous exposure to antibiotics are risk factors for MRSA in the community. MRSA in ambulatory patients is genotypically similar to nosocomial isolates and it is presumably acquired through contact with health care personnel and/or colonized/infected patients.

P738 Comparison of antibiotic resistance of the most common Gram-negative pathogens and *Staphylococcus aureus* isolated from patients with urinary or respiratory tract infections

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Objectives: Urinary tract infections (UTI) and respiratory tract infections (RTI) are the most common infections in hospitalized patients. As the most common pathogens for both kinds of infection are different, the antibiotics commonly used are not identical, resulting in different selection pressure. Therefore, it should be expected that antimicrobial resistance of bacterial species isolated from both UTI and RTI should also be different. For those species which are causing infection in both sites we compare the incidence of resistance using the data of the German Network for Antimicrobial Resistance Surveillance (GENARS).
Methods: In all laboratories involved in the GENARS-project, in the daily routine, susceptibility is measured by MIC determination. Once a week, for central evaluation these data are transmitted to the central office. Selection criteria for the query in a time frame of January 1 to November 30 in 2002 included all first isolates of a patient for the species common in both sites. Therefore, the data of the typical kinds of patient-material of the respiratory tract (sputum, tracheal secretion, bronchial secretion, pleural puncture, material from pleural biopsy, pus, bronchioalveolar lavage) and the urinary tract (urine: mid stream, bag, catheter, puncture) were evaluated.
Results: The number of cases evaluated with 80–2100 per species and antibiotic is quite high. Only those species have been evaluated which are common in both infection sites. Mainly nosocomial RTI's are included and not community-acquired infections which are usually caused by *S. pneumoniae* and *H. influenzae*. Differences in the incidence of resistant strains (see Table 1) are striking in some cases (nine-fold, Ciprofloxacin and *E. cloacae*) on a low level but may be significant also at a high level (three-fold, 45% vs. 15% in *S. aureus* with Ciprofloxacin). Although these differences are significant, they seem not to be based on a principle.

Table 1 Percentage of resistance of the same bacteria isolated from UTI and RTI (UTI/RTI)

	E. cloacae	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*P. aeruginosa*	*P. mirabilis*	*S. aureus*
Ampicillin	R	**40/49**	R	R	R	**31/39**	40/40
Piperacillin	**33/19**	**24/34**	**14/19**	**18/11**	**9/13**	**10/5**	–
Oxacillin	–	–	–	–	–	–	**31/14**
Cefaclor	R	**8/15**	**12/19**	**16/9**	R	**12/22**	**36/28**
Cefuroxime	**63/52**	8/12	**14/19**	**19/9**	R	7/**12**	–
Ciprofloxacin	**9/1**	**9/14**	4/3	3/1	13/11	5/2	**45/15**
Gentamicin	5/1	**4/10**	4/4	7/4	**10/31**	12/8	7/7
Doxycycline	**21/8**	35/34	20/24	23/5	R	R	–
Erythromycin	–	–	–	–	–	–	**25/17**

Bold letters indicate a difference between UTI and RTI of more than 5%.
R, natural resistance.
–, not done.

Conclusions: Antimicrobial resistance of the same bacterial species isolated from respiratory tract and from urinary tract of hospitalized patients in German university hospitals differs significantly. This might be due to the different therapeutical action for UTI and RTI. Thus, for the calculated therapy not only local statistics in the epidemiology of resistance has to be considered but also the site of infection.

P739 Characterization of invasive strains of vancomycin-resistant enterococci isolated in Italy, 2001–2002

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Objectives: The aim of this study was to characterize recent strains of vancomycin-resistant enterococci (VRE) isolated from patients with invasive diseases in different areas of Italy. The strains were collected over the years 2001–2002, the majority as part of the national surveillance of antibiotic resistance AR-ISS.
Methods: A total of 25 invasive VRE strains (24 from blood and 1 from CSF), were isolated in 12 different laboratories and submitted to be further characterized. Characterization included a multiplex PCR assay to detect species-specific ligases to confirm species identification and to detect the different vancomycin resistance genetic determinants (vanA, vanB, vanC1/2). Susceptibilities to different antibiotics were tested by the microdilution method using Sensititre panels. To analyze clonal relatedness among the strains, profiles of total genomic DNA digested with Sma I were obtained by pulsed-field gel electrophoresis (PFGE). The PFGE profiles were interpreted by standard criteria.
Results: The multiplex PCR analysis confirmed that out of 25 invasive VRE, 21 belonged to the species *E. faecium* and 4 to the species *E. faecalis*. All of the isolates, except one, carried the *vanA* gene, whereas a single *E. faecium* strain carried vanB. The antibiotic susceptibility assays performed on 21 VRE *E. faecium* showed that all the strains were resistant to ampicillin, the majority were also resistant to high level streptomycin (20 strains out of 21) and gentamycin (19 strains out of 21). A single strain, the vanB-positive *E. faecium*, was susceptible to both streptomycin and gentamycin. Five strains were resistant to tetracycline and one to quinupristin/dalphopristin. The results of genotyping by PFGE showed that the majority of the isolates had very similar electrophoretic profiles, differing by 1–3 bands only.
Conclusions: As in other European countries, in Italy the majority of VRE belong to the species *E. faecium* and resistance to vancomycin is mainly conferred by the gene cluster containing vanA. Interestingly, we found an isolate carrying vanB, the first such report to date in Italy. The VRE *E. faecium* strains were also resistant to several other antibiotics used to treat enterococcal infections. The PFGE analysis suggest that the invasive VRE *E. faecium* circulating in different hospitals and areas of Italy have a clonal origin.

P740 Antibiotic resistance association with age and sex in *Escherichia coli*

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Background: *Escherichia coli* antibiotic resistance is a serious and common health problem. Empiric therapy is based on local pattern of resistance. We studied the resistance relationship with patient age and sex.
Methods: 1157 patients with a positive urine culture for *E. coli* within February 2001 and October 2001 retrospective analysis (only one isolate from each patient). The identification and susceptibility tests were done with MicroScan WalkAway. We evaluated Ampicilin (Ap), Amoxycilin-Clavulanate (Amc), Gentamicin (Gn), Cefotaxime (Ctx), Cotrimoxazol (Sxt) and Ciprofloxacin (Cp) resistance. Results were compared by the Chi-square or

Table 1

	Univariate			Logistic regression			
Antibiotic	%R	Sex (*P*)	Age (*P*)	Males OR	IC 95%	>40 years OR	IC 95%
Ap	59.8	0.054	0.213	1.32	0.99–1.77	1.1	0.88–1.45
Amc	10.3	0.66	0.001	1.1	0.7–1.7	1.8	1.16–2.8
Ctx	1.7	0.83	0.022	0.88	0.29–2.67	2	0.67–6.1
Gn	6.1	0.05	0	1.7	1.0–2.9	4.9	2.2–10.7
Cp	21	0.006	0	1.6	1.14–2.23	4	2.76–6.04
Sxt	29.3	0.37	0	1.1	0.8–1.5	1.5	1.1–2

Student *t*-test as appropriate. The association between age (being older than 40 years old) and sex with antibiotic resistance was evaluated by logistic regression (LR) models.
Results: Median age was 52 years (0–99), 78% were female (Table 1).
By univariate analysis, antibiotic resistance was associated to being older in all the cases except with Ap. Cp and Gm resistance were associated with sex (27.3% males vs. 19.4% females, 8.7% males vs. 5.3% females, respectively). We also found a strong correlation among the resistance to different antibiotics. The multiresistance isolates were frequent.
By LR patients older than 40 years old were four to five times more likely to present an isolate resistant to Cp or Gn, and these isolates were more frequent in males (OR 1.6 and 1.7).
Conclusions: There seem to be minor variations in the resistance to beta-lactam antibiotics with respect patients sex and age, but these variations were significant in the resistance to Gn and Cp. This fact may reflect a more stable *E. coli* population with resistance to beta-lactam antibiotics meanwhile acquired resistance to Gn or Cp may increase faster by antibiotic pressure. These variables may be useful in prediction models for empiric therapies.

P741 The short- and long-term impact of hospitalization on the colonization of the lower gastrointestinal tract with antibiotic-resistant bacteria

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Objectives: The fecal flora represents an important source of potential pathogens and a large reservoir of resistance genes, able to move to susceptible bacteria of the same or other species. Hospitalization could alter the fecal flora due to selection and/or horizontal dissemination of resistant bacteria. The present study was prospectively designed to (1) assess whether hospitalization leads to the acquisition of resistant strains in the fecal flora, (2) define the risk-factors associated with colonization with resistant strains, and (3) examine whether colonization disappears after discharge.
Methods: Study population consisted of patients admitted for any reason to a medical ward. In our department, strict policies for the containment of antibiotic resistance, including hand hygiene, are implemented. A stool specimen was collected using a rectal swab (Portagerm Amies Agar, Biomerieux) on admission, in 7–10 days, before discharge and at follow-up. Information on several possible risk factors was also recorded. Swabs were plated onto agar plates containing each (in μg/mL): ceftazidime (2 and 8), ciprofloxacin (2), cefepime (8), imipenem (4), oxacillin (6) + 2% NaCl and vancomycin (6). Isolates were submitted to identification and susceptibility testing.
Results: Of 140 patients enrolled, 32 (22.9%) were colonized with new resistant isolate(s) during hospitalization. A total of 36 different strains were isolated, the most prevalent being *E. coli* ciprofloxacin and/or ceftazidime-resistant. Fifteen patients (46.9%) received antibiotics whereas 2 (6.3%) shared the room with another colonized patient. For seven patients a median follow-up of 6 months was available and only two retained a resistant isolate.
Conclusions: In a setting where antibiotic resistance policies were implemented, fecal colonization with resistant bacteria was 22.9%, antibiotic consumption being probably the most important risk factor. The study is still ongoing in order to assess the long-term colonization rate.

P742 Dissemination of structurally related class I integrons among *Acinetobacter baumannii* clones

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Objectives: To investigate the relationship between aminoglycoside resistance genes and class 1 integrons and clonality of multiresistant *A. baumannii* strains from the Czech Republic.
Methods: Seventy epidemiologically unrelated multiresistant strains of *A. baumannii* isolated in Czech hospitals between 1991 and 2002, 13 reference strains of epidemic clones I and II from NW Europe (Dijkshoorn L et al. J Clin Microbiol 1996; 34: 1519–25) and a control group of 15 susceptible Czech strains were investigated. The strains were studied by AFLP fingerprinting and *Hin*dIII/*Hin*cII ribotyping and screened for the presence of seven aminoglycoside resistance genes and class 1 integrons by PCR. PCR mapping was used to study the content and order of integron-associated gene cassettes.
Results: AFLP fingerprinting and ribotyping classified the Czech multiresistant strains into clone I ($n = 41$), clone II ($n = 21$) and a heterogeneous group of other strains ($n = 8$). The susceptible strains had heterogeneous genotypes distinct from those of clone I or II. Aminoglycoside resistance genes aac(3)-Ia, aph(3′)-Ia, aph(3′)-VIa, ant(2″)-Ia and ant(3″)-Ia were present in 13 different combinations, some of which were found in both clones. Class 1 integrons were detected in most clones I and II strains and were classified into two types according to size (3.0 and 2.5 kb) and structure of their internal variable regions. The two integron types contained aac(3)-Ia and ant(3″)-Ia cassettes and were structurally highly related to each other as indicated by the restriction patterns and positions of gene cassettes. The integrons of the two types were found both in clones I and II strains including Czech and NW European strains.
Conclusions: The Czech multiresistant *A. baumannii* strains belonged almost exclusively to the two clonal lineages previously recognized in a set of strains from NW European hospitals. Both the intraclonal diversity of aminoglycoside resistance genes and the presence of the same resistance genes and integron structures in clonally distinct strains are indicative of horizontal gene transfer. The data suggest that the structure of the integrons remains stable over a long period of time.

In vitro susceptibility testing

P743 Accuracy of four susceptibility methods for detection of oxacillin resistance in *Staphylococcus aureus*

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Oxacillin-resistant *Staphylococcus aureus* (MRSA) is responsible for an increasing number nosocomial- and, more recently, community-acquired infections. Effective control measures for reducing the hospital reservoir of MRSA depend, basically, on correct identifications of these strains. The most common methods currently used for identifying oxacillin-resistance in many clinical laboratories are susceptibility tests. The performance of these tests has been erratic because the expression of resistance is variable and, commonly, heterogeneous within strains. Using a set of 103 nosocomial strains of *S. aureus*, four routine methods were evaluated (disk diffusion, agar dilution, oxacillin agar screen, and Vitek GPS-101 card) by using the presence of the *mecA* gene, as detected by PCR, as the 'gold standard'. The susceptibility tests were carried out as recommended by the NCCLS. Among all the isolates, 36 were identified as mecA-positive, and the remained 67 were identified as mecA-negative. The results of the susceptibility tests (oxacillin-resistant/oxacillin-susceptible) were as follows: disk diffusion, 33/70; agar dilution, 41/62; oxacillin agar screen, 34/69; and Vitek, 45/58. The percentages of correct results (% sensitivity/% specificity) were: disk diffusion, 81/94; agar dilution, 97/91; oxacillin agar screen, 94/100; and Vitek GPS-101 card, 97/85. Ten isolates, negative for the mecA gene by PCR, were recognized by at least one phenotypic method as oxacillin-resistant. Further evaluation of these organisms reveled that only two were susceptible to amoxacillin-clavulanic acid. Hyperproduction of beta-lactamase was considered the most likely cause of false positive result in these two strains, and could not be determined in the other eight. Only two strains mecA-positive were incorrectly identified as oxacillin-negative by the oxacillin agar screen, although spot inoculation method with a 1 μL loopful of a 0.5 McFarland suspension were used to improve sensitivity/specificity. Assay to determine the cause of this discrepancy were not done. As shown in this and other studies, no phenotypic method is completely reliable for the detection of oxacillin resistance in *S. aureus*. The oxacillin screen test is the most accurate test and approaches the accuracy of PCR. It should be considered in association with other susceptibility method to maximize the ability to correctly detect oxacillin-susceptibility in *S. aureus*.

P744 Evaluation of a novel medium for the screening of methicillin-resistant *Staphylococcus aureus* among hospitalized patients

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A novel medium, the oxacillin-resistant screening agar medium (ORSA), was evaluated for the screening of MRSA in the hospital setting. Screening swabs (nose, throat, perineal, and infected sites) were inoculated onto this new ORSA medium and in an enrichment broth (Muhler-Hinton supplemented with NaCl and oxacillin). After 24 h of incubation, the enrichment broth was sub cultured onto one ORSA plate and one lipovitellin Chapman salt agar plate. The sensitivity to detect MRSA was calculated for each medium alone or in combination. A low sensitivity (83%) was obtained when the ORSA medium was used alone as a primary culture. Among the 414 blue colonies observed on ORSA plates, 47% were found to be MRSA, 41% were coagulase-negative straphylococci, 6% were *Enterococcus* species, and 2% were methicillin-sensitive *S. aureus*. The optimal incubation time of the ORSA plates was evaluated: on primo-culture, 38% of the blues colonies were visible only after 48 h of incubation (not seen at 24 h of incubation), whereas 94% of the colonies were visible already at 24 h when ORSA plates were used for subcultures. In conclusion, the advantage of the novel ORSA medium is the ease of recognition of mannitol-fermenting bacteria, but further identification tests are needed to confirm the diagnostic of MRSA. An enrichment broth is still needed to ensure a good sensitivity to recover MRSA, and an incubation time of 48 h is required for the primo-culture of the ORSA medium.

P745 Comparison of methods used for the detection of methicillin-resistant *Staphylococcus aureus*

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Objective: To determine the most accurate method for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA).
Methods: Methicillin (oxacillin) susceptibility of 111 *S. aureus* isolates, which were recovered from various clinical specimens were studied by four different methods: (1) NCCLS disk diffusion test; (2) Susceptibility determination by the Vitek GPS 101 cards (BioMerieux; France); (3) Direct detection of PBP2a with MRSA-Screen (Denka Seiken; Japan) test; (4) mecA gene detection by PCR.
Results: The number of isolates found as resistant to methicillin was 80 for the disk diffusion test, 85 for the Vitek system, 81 for the MRSA-Screen test, and 76 for the mecA gene analysis. According to mecA gene analysis, sensitivity and specificity of disk diffusion, Vitek, and MRSA-Screen tests are 100 and 90%, 100% and 80%; 100 and 88%, respectively. When the isolates which had shown discrepancies with different methods were re-studied, it was observed that all results were in complete agreement with the MRSA-Screen test.
Conclusion: NCCLS disk diffusion test and Vitek system, providing that all recommendations were followed carefully, are suitable tests to be used in routine laboratories to detect MRSA isolates, whereas the MRSA-Screen test can be preferred as a verification tool due to its being a fast, easy and dependable method.

P746 Detection of methicillin resistance in *S. aureus* – a disc with cefoxitin 30 g is superior to oxacillin 1 g

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Background: Accurate phenotypic detection of methicillin resistance in *S. aureus* has proven very difficult, especially of strains with heterogeneous and low-level methicillin resistance. Recently the use of a cefoxitin 30 μg disc on Mueller Hinton agar and confluent growth was shown to perform very well. In this study we investigated the use of a cefoxitin 30 μg disk on standard IsoSensitest Agar using a semi confluent inoculum and compared it to the method recommended by the Swedish Reference Group of Antibiotics (SRGA).
Material and methods: A total of 456 *S. aureus* strains comprising 181 mecA positive *S. aureus* and 275 consecutive *S. aureus* routine isolates were investigated. A large number of MRSA phenotypes, including heterogeneously and low-level resistant variants, were included. All strains were investigated with a 30 μg cefoxitin disk (Oxoid, Basingstoke, UK), on standard IsoSensitest Agar (Oxoid), using semi confluent growth and overnight incubation in ambient air at 35–37°C as well as by a 1 μg oxacillin disk (Oxoid) on IsoSensitest Agar supplemented with 5% defibrinated horse blood, confluent growth and full 24 h incubation in ambient air at 30°C (SRGA recommended method). The methods were evaluated using a best-fit breakpoint for cefoxitin and the published SRGA breakpoints for oxacillin, respectively.
Results and discussion: All MRSA but only two MSSA isolates exhibited cefoxitin zones of <29 mm resulting in a sensitivity of 100% and a specificity of >99%. The corresponding values for the SRGA recommended method was 77 and 99%. Since there was considerable overlap between MRSA and MSSA, changing the breakpoint for the SRGA method could not solve the problem. Apart from the increased sensitivity and specificity of the cefoxitin method it has the advantage of allowing the laboratory to utilize the same plate, inoculum, incubation atmosphere and time used for susceptibility testing of *S. aureus* against other antibiotics.
Conclusion: A cefoxitin 30-μg disk is highly accurate for the detection of methicillin resistance in *S. aureus*. We suggest a tentative breakpoint of $R < 29$ mm and $S \geq 29$ mm on IsoSensitest Agar using semi confluent growth, overnight incubation in ambient air at 35–37°C. We suggest that this method should replace the method currently recommended by the SRGA.

P747 Evaluating different methodologies to detect glycopeptide intermediately susceptible *Staphylococcus aureus* – the Dutch experience

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Background: Vancomycin resistance will have a major impact on the treatment of *Staphylococcus aureus* infections around the world. To determine the incidence of hGISA/GISA (and its consequent clinical impact) methods that will reliably and reproducibly discriminate these resistant phenotypes from normal MRSA must be defined. The present study was a separate part within and adapted from a Pan-European/American study protocol.
Objective: Aim of the study was to assess and compare the ability to discriminate vancomycin-susceptible MRSA from hGISA/GISA phenotypes and the intra- and interlaboratory reproducibility of agar screening plates and the *E*-test method.
Methods: At total of 100 isolates (10 MRSA, 9 hGISA and 6 GISA – as determined by PAP-AUC ratio, each strain in quadruplets) were sent to eight Dutch study centres. Investigators were blinded with regard the isolates' phenotype. All strains were PFGE typed to eliminate clonal duplication. Each participant performed test for vancomycin and teicoplanin resistance by three agar screening methods (BHI + 6 μg/mL vancomycin, MH + 5 μg/mL vancomycin, MH + 5 μg/mL teicoplanin) and the *E*-test macro-method using 2.0 McFarland. All media were centrally supplied. *E*-test's were interpreted as positive when vancomycin was ≥8 and teicoplanin ≥12 or ≥8 (combined results) (Table 1).

Table 1

Results	BHI-van	MH-van	MH-teico	*E*-test
MRSA correctly identified (%)				
MRSA	97.8	99.1	94.4	84.1
hGISA (%)				
Sensitivity	3.1	0.7	64.9	80.6
Specificity	94.4	99.7	94.4	83.4
GISA (%)				
Sensitivity	85.9	50.5	95.8	99.5
Specificity	98.4	99.7	94.4	83.4

*Final results may differ due to the still ongoing retesting of hGISA with regard to their stability to express this phenotype.

Conclusions: Independent of the phenotype of the isolates and the method used for detection, the specificity was generally high, especially with the agar-screening methods. More importantly, the sensitivity (looking for the true

positive results) varied between the different methods and especially between the different phenotypes. BHI-vancomycin- and MH-vancomycin-screening agars completely lacked the ability to detect hGISA. The use of MH-teicoplanin screening agar and the teicoplanin *E*-test, seemed to be the most efficient methods to detect hGISA/GISA, with *E*-test achieving the highest sensitivity. The intra- and interlaboratory reproducibility of all methods was very high.

P748 BDXpert™ system: implementing NCCLS and SFM rules in a knowledge base for interpretation of antibiotic susceptibility testing results

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Objectives: The National Committee for Clinical Laboratory Standards (NCCLS, USA) and Comite De L'Antibiogramme De La Societe Francaise De Microbiologie (SFM, France) regularly publish standards for performance and interpretation of antimicrobial susceptibility testing (AST) results. These documents contain information that laboratories can use to assist clinicians in selection of appropriate antimicrobial agents for treating bacterial infections. Effective communication of this information is critical for providing proper patient cares. The BDXpert System knowledge base has been developed for use in the BD Phoenix™ Automated Microbiology System and BD Epi-Center™ System to enhance the interpretation of AST test results as recommended by NCCLS or SFM. This study evaluated the functionalities of the BDXpert System.
Methods: Statements listed in the NCCLS M100-S12 and SFM Report 2000–2001 documents were converted into expert rules. Special rules were also constructed for the detection of the following resistant markers: extended-spectrum beta-lactamase (ESBL), methicillin-resistance staphylococci (MRS), vancomycin resistance enterococci, high-level gentamicin resistance and streptomycin resistance in enterococci, beta-lactamase production in staphylococci (S-BL), and vancomycin intermediate staphylococci. Associated actions were implemented as specified in the standard. A total of 222 Gram-negative or Gram-positive isolates with MIC results were used to evaluate the BDXpert System.
Results: Phoenix MIC values were interpreted according to the breakpoints listed in the standards for the appropriate organism-drug combinations and SIR interpretations generated. BDXpert rules were applied and applicable expert messages were displayed. When 33 isolates of ESBL were detected, selected beta-lactams with susceptible (S) or intermediate (I) results were changed to resistant (R) with the NCCLS as the standard. When SFM criteria were applied to the same isolates, selected beta-lactams with S results were changed to I. For 35 MRS isolates, all beta-lactams with S or I were changed to R both with NCCLS and SFM. For 37 S-BL strains, all hydrolysable penicillins were changed to R.
Conclusions: The BDXpert System has proven to be a reliable system that can assist laboratory reporting and proper interpretation of AST results. Special messages can be used to communicate timely and accurate information to clinicians for proper therapy of infections.

P749 POLMICRO 2002 – national external quality assessment scheme in microbiology

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Objectives: The objective of this study was to analyze the results of Polish National External Quality Assurance Scheme in Microbiology, POLMICRO 2002
Methods: Ten bacterial isolates representing key antimicrobial-resistance phenotypes were distributed to 370 laboratories in Poland. Each isolate was blind-coded and laboratories were asked to identify the strains to the species level and test for antimicrobial susceptibility. Participants received data collection sheet, which contained questions about routine work of the laboratory, e.g. method used, source of media and reagents, interpretive criteria used. The following strains were distributed: *Enterococcus faecalis* (vancomycin-resistant VanB, HLAR), *E. faecium* (vancomycin-susceptible), *Staphylococcus aureus* (methicillin-resistant), *S. aureus* (methicillin-susceptible), *Escherichia coli* and *Klebsiella pneumoniae* (extended spectrum β-lactamase producer). The organisms were tested multiple times at the Centre of Quality Control in Microbiology by disk diffusion and agar dilution to establish the reference MIC and disk diffusion values. In some cases PCR assays for mecA, vanA and vanB were used to confirm the resistance mechanisms.
Results: Of the 370 laboratories volunteered to participate in the study, all (100%) completed the proficiency test and returned a completed data report sheet. Almost 96% laboratories were able to identify isolates to the species level adequately. Most of the laboratories were able to detect methicillin resistance in *S. aureus*, vancomycin resistance in *E. faecalis* and resistance to extended-spectrum cephalosporins in *E. coli* and *K. pneumoniae*. The most difficult phenotype to detect was high-level aminoglycoside resistance in *E. faecalis*.
Conclusions: The majority of laboratories proved to be reliable and keeping up with high standards of microbiological diagnostics, which was confirmed by their capabilities to accurately identify species and determine susceptibility of clinically relevant strains.

P750 Investigation of the synergistic effects of combinations of beta-lactam and fluoroquinolone or aminoglycoside against clinical isolates of *Pseudomonas* spp. with *E*-test technology

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Objective: The aim of the study was to investigate the applicability of *E*-test technology for antibiotic combination testing in clinical routine work.
Methods: The *E*-test was used by superimposing the gradients of various antibiotics to detect potential synergy between beta-lactam antibiotics (ceftazidime, piperacillin, or imipenem) and fluoroquinolones (ciprofloxacin) or aminoglycosides (amikacin or tobramycin) against 110 clinical isolates of *Pseudomonas* spp. Interpretation of the results was based on calculation of the fractional inhibitory concentration (FIC) index for all isolates.
Results: Synergistic, additive or indifferent effects were observed as concerns the *Pseudomonas* spp., depending on the isolates; antagonism was not seen. In 10 of 30 ciprofloxacin-resistant or multiresistant *Pseudomonas/Burkholderia* isolates (33.3%), synergy was seen between ceftazidime and ciprofloxacin or piperacillin and amikacin combinations. Other combinations, such as piperacillin and ciprofloxacin (23.3%), and imipenem or ceftazidime and ciprofloxacin (16.6%) combinations displayed synergy. Imipenem and tobramycin were synergistic against two isolates (6.6%). For one ciprofloxacin-resistant *Burkholderia cepacia* isolate, synergy was observed between ceftazidime and ciprofloxacin and between piperacillin and amikacin.
Conclusion: The Etest is an easy and useful MIC method for clinical laboratories to utilize for the combination testing of various antibiotics. Calculation of the FIC indices provides an effiecient means of interpreting the results.

P751 Erythromycin zone breakpoints should be changed in susceptibility testing of *Streptococcus pyogenes*

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Objective: Macrolide resistance in *Streptococcus pyogenes* (SP) is increasing worldwide, and use of macrolides in treatment of infections where SP could be the pathological organism has become standard in many countries. This calls for a reliable susceptibility testing method and accurate zone breakpoints
Methods: We performed susceptibility testing with 87 macrolide resistant (MR) and 50 susceptible SP according to three different standards: the NCCLS, The Swedish Reference Group for Antibiotics (SRGA) and the Rosco tablet diffusion. MIC was determined for MRSP by *E*-test.
Results: Susceptibility testing by disc diffusion according to NCCLS standards on 87 MRSP found 8 isolates (9%) as intermediate susceptible. Disc diffusion according to SRGA on 87 MRSP found 10 (11%) as intermediate susceptible and 5 (6%) as susceptible. Tablet diffusion according to Rosco on 87 MRSP; found 16 (18%) intermediate susceptible and two (2%) as susceptible. There were no errors in the determination of the susceptible isolates for any of the methods. *E*-test found MIC for the MRSP ranging from 2 to >256.
Conclusion: Zone breakpoints should be changed: by Rosco method resistance should be defined as equal or smaller than 28 mm, by SRGA method resistance should be defined as equal or smaller than 24 mm and by NCCLS method resistance should be defined as equal or smaller than 20 mm.

P752 Antimicrobial susceptibility testing of streptococci and *Haemophilus influenzae* using a medium with defibrinated horse blood and NAD

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Objectives: Antimicrobial susceptibility testing (AST) of fastidious organisms requires that base media such as ISA (Oxoid Ltd), PDM (AB Biodisk) or MH (various) are supplemented with nutritionals to permit good growth. The SRGA-M (Swedish Reference Group for Antibiotics subcommittee on methodology) recommends the addition of defibrinated horse blood for AST of streptococci and pneumococci and of 1% hemoglobin (Oxoid) and 1% IsoVitaleX (BBL) for AST of *Haemophilus influenzae.* The BSAC (British Society of Antimicrobial Chemotherapy) working party on AST recommends the addition of defibrinated horse blood and NAD for *H. influenzae* and suggests that the same plate may be used for streptococci, pneumococci and *Neisseria.* The present study compared the two recommendations to find out if the SRGA-M recommended plate for *H. influenzae* could be scrapped.
Methods: Laboratory reference strains and clinical isolates (collected during 1997–2002) were examined by the disc diffusion test. The plates were read after 20 h incubation at 37°C in 5% CO_2. Bacterial growth, together with the diameter and appearance of the inhibition zones was noted. The following species were included in the study: *H. influenzae*, *Moraxella catarrhalis*, *Streptococcus agalactiae*, *S. pyogenes*, *S. pneumoniae* and streptococci of Lancefield groups C and G.
Results: With two exceptions the results on the two media were identical. For *H. influenzae* the inhibition zones for phenoxymethylpencillin was smaller (−3 mm) on the NAD-containing medium. For *M. catarrhalis* cotrimoxazole produced pronouncedly larger zones on the NAD-medium.
Conclusion: The ISA (Oxoid) medium containing defibrinated horse blood and NAD can be used for AST of streptococci, pneumococci, *H. influenzae* and *M. catarrhalis*. It can replace the medium with 1% hemoglobin and 1% IsoVitaleX hitherto recommended by the SRGA-M for *H. influenzae.* This simplifies the production and quality control of AST media. With two exceptions (*H. influenzae* and phenoxymethylpencillin and *M. catarrhalis* and cotrimoxazole) no adjustments are necessary to zone diameter breakpoints.

P753 Antimicrobial susceptibility testing of beta-hemolytic and viridans group streptococci using the BD Phoenix™ Automated Microbiology System

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Objectives: Viridans (VS) and beta-hemolytic (BS) streptococci are important agents of infections such as sepsis, menigitidis, and endocarditis. Increase in frequency of penicillin resistant VS and macrolide resistant BS from clinical isolates have been reported. Resistance testing among this important group of organisms is difficult to test in a routine laboratory using traditional overnight broth dilution systems. The performance of the Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) with the newly developed Streptococcal AST panel was evaluated for ability to detect resistance for 12 antibiotics in VS and BS. Results from these antibiotics; Penicillin (P), Erythromycin (E), Cefotaxime (CTX), Ceftriaxone (CRO), Chloramphenicol (C), Gatifloxacin (GAT), Levofloxacin (LVX), Meropenem (MEM), Ofloxacin (OFX), Quinupristin/Dalfopristin (SYN), Tetracycline (TE), and Vancomycin (VA) are evaluated in this study.
Methods: A total of 86 BS and 42 VS were tested, including recent clinical isolates and previously characterized genotype and phenotype resistant strains. All testing was performed in parallel using the Phoenix System and NCCLS recommended standard broth microdilution (SBM) method. Phoenix and SBM were analyzed for essential accord (EA) and categorical agreement (CA) based on NCCLS established breakpoints.
Results: EA for each drug was greater than or equal to 90% except CTX, GAT and TE which are at 89, 86, and 80%, respectively. CA for each drug was greater than or equal to 93%. Very Major Errors and Major Errors were less than 1.5% for all drugs except for E and TE. E resulted in a VME of 4.8% due to 2 out of 42 resistance strains and TE showed 1.6% ME based on 1 out of 63 susceptible strains. The overall average time to result (TTR) for all drugs using the Phoenix System was 8.4 h.
Conclusions: This study indicates that the Phoenix Streptococcal AST panel provides rapid, reliable MIC determination for VS and BS. This important group of organisms is showing increased resistance and is typically difficult to test using traditional overnight broth dilution systems. The Phoenix System provides a new improved method for susceptibility testing of streptococci.

P754 Fluoroquinolone susceptibility testing of pneumococci using the BD Phoenix™ Automated Microbiology System

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Objectives: *Streptococcus pneumoniae* (SP) is one of the most common causative agent for community-acquired pneumonia and acute exacerbations of chronic bronchitis. The increase in frequency of resistance to penicillin, extended spectrum cephalosporins, and macrolides have made fluoroquinolones an important choice of empiric treatment. The Phoenix™ Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) was evaluated for its ability to detect resistance to fluoroqinolones in SP.
Methods: Drugs evaluated include Levofloxacin, Moxifloxacin, Ofloxacin, Gatifloxacin, Gemifloxacin, and Garenoxacin. A total of 190 SP were selected for the evaluation. Selected strains include recent clinical isolates and phenotypically/genotypically characterized strains. All testing was performed in parallel using the Phoenix System and the NCCLS recommended standard broth microdilution (SBM) method supplemented with lysed horse blood. Phoenix and SBM were analyzed for essential accord (EA) and categorical agreement (CA) based on NCCLS established or manufacturer recommended breakpoints.
Results: Essential accord for each drug was greater than or equal to 97%. CA for each drug was greater than or equal to 94%. Very major errors are at 0% for all drugs tested. Major errors are at 0% for all drugs except for Moxifloxacin (0.6%) and Garenoxacin (2.7%). The average time to result (TTR) based on the Phoenix system for each drug ranged from 7.6 to 9.4 h.
Conclusions: This study indicates that the Phoenix System provides rapid, reliable MIC determination for SP against a broad selection of fluoroquinolones including the newly developed desfluoroquinolone, Garenoxacin.

P755 Evaluation of ID and AST with 241 strains of *Pseudomonas aeruginosa* comparing the PHOENIX™ and the VITEK (R) 2 system

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Objectives: *Pseudomonas aeruginosa* is a very common but sometimes difficult organism for rapid automated systems to accurately identify (ID) and perform antimicrobial susceptibility testing (AST). We compared the accuracy of two such systems the VITEK 2 (V2) (bioMérieux, Marcy L'Etoile, France) with the Phoenix (PHX) system (BD Diagnostic Systems, Sparks, USA) with 241 *Pseudomonas aeruginosa* strains.
Methods: A total of 241 clinical isolates of *P. aeruginosa* was tested, including a significant number of strains resistant to extended spectrum cephalosporins, carbapenems, aminoglycosides and fluoroquinolones. The isolates were collected in the routine lab of Laboratory Group Heidelberg from various specimens. PHX combination ID and AST panel NMIC/ID-5 were used, and for V2 the Gram-negative ID-GNB and the AST card AST-GN09 were used. All tests were performed following the manufactures recommended procedures. For ID reference the ID 32 GN strips (BioMérieux) were used. Discrepant ID results were retested in duplicate. For AST the following antibiotics were evaluated: gentamicin, tobramycin, amikacin, ciprofloxacin, levofloxacin, piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, and meropenem. The results of AST were compared with the reference broth microdilution method (Biotest AG, Dreieich, Germany) according to NCCLS recommendations. Discrepant results were repeated in duplicate, data analysis was performed following the FDA guidance document.
Results: The V2 system correctly identified 99% of the species, the PHX system 100%. With regard to AST, overall category agreement (CA) for the V2 was 90.6%, and 90.2% for the PHX instrument (range PHX: 62.7% (cefepime)- 98.8% (piperacillin, amikacin); range V2: 77.9% (meropenem)-99.2% (piperacillin)). Major error rate for the V2 and PHX were 0.4% (6), and 1.3% (20), while the very major error rate were 2.0% (12) and 1,.0% (6),

respectively. The majority of VME for both systems were seen with Meropenem, 6 with V2 and 4 with PHX.
Conclusions: The V2 and the PHX systems, showed good performance concerning ID and AST of *P. aeruginosa* isolates, considering the challenging characteristics of this species for susceptibility testing methods.

P756 A multicentre evaluation of the VITEK 2 Advance Expert System (AES) using selected bacteria harboring characterized mechanisms of resistance to antibiotics

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Objectives: To evaluate the performance of the VITEK 2 AES software for the detection of difficult resistance phenotypes in a set of well-characterized bacterial strains.
Methods: Six laboratories tested a collection of 60 reference strains (40 Gram-negative bacilli (32 Enterobacteriaceae, 8 *Pseudomonas aeruginosa*, 12 staphylococci, 8 enterococci) comparatively by the VITEK 2 system and by their own routine testing methods (disc diffusion) against 10–20 antimicrobial agents. The strains were selected on the basis that they harbored challenging and important resistance genotypes.
Results: Interpretive reading by the VITEK 2 AES achieved full agreement with genotype data or identified the correct mechanisms as one or two other possibilities in 82, 86, and 98% of the Gram-negative bacilli, staphylococci, and enterococci strains, respectively. By contrast, the correct resistance phenotypes were identified in only 39, 56, and 20% for the same three groups of organisms by the investigators when using their own routine method. The ability to recognize resistance mechanisms in the set of strains varied widely from one centre to another. The most frequent reasons accounting for the failure of detecting resistance by the investigators included either inappropriate selection of the agents tested, absence of recognition of phenotypes with low expression of resistance (e.g. vanB, hetero resistant VISA, ESBLs, quinolone efflux resistance mechanisms. . .) or the absence of interpretive reading of results. Clinically important mechanisms inferred with >90% agreement with reference data by the VITEK 2 AES included methicillin resistance in staphylococci, high-level aminoglycoside resistance in Gram-positive cocci, VanA, VanB, and VanC phenotypes in enterococci, ESBLs, AmpC cephalosporinases and various types of penicillinases in Enterobacteriaceae and *P. aeruginosa*.
Conclusions: The VITEK 2 AES system appears as a reliable tool and is clearly superior to locally used routine methods for the detection and interpretive reading of clinically important mechanisms of resistance.

P757 Identification and susceptibility testing of vancomycin-resistant enterococci using automated systems: VITEK2 and Phoenix™

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Objective: Infections caused by vancomycin resistant enterococci (VRE) are difficult to treat and require infection control measures. The fact that VRE-infections are still uncommon in The Netherlands, makes their fast and reliable detection even more important. We evaluated the performance of two automated systems with respect to identification and susceptibility testing of VRE.
Material and methods: Fifty-five isolates of molecular identified VRE (7 *Enterococcus casseliflavus* ECCA, 2 *E. faecalis* ECFA, 19 *E. faecium* ECFI, and 27 *E. gallinarum* ECGA) with known VAN status (17 VanA, 4 VanB, 34 VanC1/2/3) were tested for identification and vancomycin and teicoplanin MIC on the VITEK2 (BioMerieux) and Phoenix™ (BectonDickinson) system.
Results: Identification: None of the systems could differentiate between ECCA/ECGA. Simple further tests were necessary to obtain the correct identification and were advised by the systems software. VITEK2 and Phoenix™ correctly identified the strains as follows: ECCA 7/7 and 6/7, ECFA 2/2 and 2/2, ECFI 12/19 and 16/19, ECGA 26/27, and 27/27, respectively. Susceptibility: Vancomycin MICs were correctly measured by both systems in all strains (55 of 55). In 3 of 55 strains, TEC MICs measured by VITEK2 were too low, suggesting VanB resistence in VanA positive strains. The same was true for 2 strains tested in Phoenix™. Additionally, Phoenix™ suggested a VanA type in one VanC strain.
Conclusion: In this study, VITEK2 and Phoenix™ both detect VRE fast and reliable.

P758 Performance of identification and susceptibility testing of automated systems VITEK2 and Phoenix™ of *Pseudomonas aeruginosa* from cystic fibrosis patients

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Objective: *Pseudomonas aeruginosa* is the most frequent colonizing and infecting pathogen in patients with cystic fibrosis (CF). Identification and susceptibility testing of those isolates is time-consuming thus a fast and reliable method would be desirable.
Material and methods: We tested 30 strains of *P. aeruginosa* (13 nonmucoid (nm) and 17 mucoid (m)) isolated from CF patients. The strains were identified by conventional methods and MICs (tobramycin (TOB), ceftazidime (CAZ), meropenem (MEM), ciprofloxacin (CIP)) were determined by an agar-dilution technique and interpreted according to NCCLS guidelines. Identification and susceptibility testing were then performed on the automated systems VITEK2 (BioMerieux) and Phoenix™ (BectonDickinson) according to the manufacturers guidelines.
Results: Identification: The VITEK2 system identified 19/30 (57%) strains correctly. For 10 (4 nm, 6 m) strains, further tests were necessary to obtain the correct identification. One strain remained unidentified (nm). The Phoenix™ identified 27 strains (81%) correctly. Three strains (2 nm, 1 m) were identified only to the species level. One of these strains (m) had been identified correctly by VITEK2. Susceptibility testing: the rate of minor, major and very major errors for the VITEK2 and Phoenix™ were as shown in Table 1.

Table 1

	VITEK2				Phoenix™			
	TOB	CAZ	MEM	CIP	TOB	CAZ	MEM	CIP
Minor	2	2	2	9	2	2	2	7
Major	0	0	0	1	0	0	0	1
Vary major	1	0	0	1	0	0	0	1
Not determined	5	5	5	5	3	4	5	4

Conclusion: In this study, the ability to identify of *P. aeruginosa* was more reliable with Phoenix™ than with VITEK2. Major drawback with both systems was the high number of strains for which no susceptibility testing was done and the high number of errors when testing CIP.

P759 A multicentre comparison between the VITEK 2 and the NCCLS disk diffusion susceptibility testing

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Objectives: To compare the VITEK 2, a new automated instrument for rapid (results the same day) antimicrobial susceptibility testing, with the NCCLS disk diffusion method with routine strains.
Methods: Six laboratories participated in this study, five hospital laboratories and one private laboratory. Five hundred and seventy-two strains were tested (295 Enterobacteriaceae belonging to 19 species, 69 non-fermenters belonging to 2 species, 131 *Staphylococcus* spp., 59 *Enterococcus* spp., and 18 *Streptococcus pneumoniae*). The strains were isolated from different clinical relevant specimens and were selected at random. The number of *Escherichia coli* was limited to 30 per center. The reagents and culture media (BioMérieux) were the same in all the labs. A difference of one category between the two methods was considered a minor discordance, a difference of two categories was a major discordance.
Results: The overall degree of agreement between the two methods was 88.5% (3646 paired results on 4121). There were 8.6% minor discordances and 2.9% major discordances between NCCLS-disk and VITEK 2. The best score of an individual lab was 92.9% full agreement, the worst was 82.5%. The three labs with the lowest overall agreement disagreed mainly with *E. coli* and *Pseudomonas aeruginosa*. The antibiotics with the lowest concordance were

amoxiclav with the Enterobacteriaceae and gentamicin high level with the enterococci.
Conclusions: The VITEK 2 seems well suited for use as a routine method of antimicrobial susceptibility testing.

P760 Time-to-result for antimicrobial susceptibility testing using the VITEK®2 instrument in a routine laboratory

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Objectives: The basic goal of automated bacterial susceptibility test systems is to produce reliable results in rapid time, preferably on the same workday. We collected the time to result data of the VITEK 2 system (BioMérieux, Marcy l'Etoile, France) for antimicrobial susceptibility testing (AST) in our routine laboratory. The evaluation was performed from January 2002 to February 2002 comprising 3134 fresh clinical isolates belonging either to Gram-positive cocci (staphylococci and enterococci) or Gram-negative rods (Enterobacteriaceae, and non-fermenters).
Methods: A total of 3134 fresh clinical isolates were routinely tested from blood cultures, wound-, vaginal- and respiratory tract specimens including: *Staphylococcus aureus* (336), Coagulase negative staphylococci (CNS) (481), *Enterococcus* spp. (537), Enterobacteriaceae (1525), *Pseudomonas aeruginosa* (200) and others (55). AST for Gram-negative rods was performed using the VITEK 2 AST-N021 card, for staphylococci and enterococci using the AST-P526 card, respectively. Concerning time to result the following data were calculated: the mean values ± standard deviation (SD), the ranges, and the time when 50, 90, and 95% (T50, T90, T95) of the results were available.
Results: The mean time to result for all bacterial isolates tested was 8.1 ± 2.3 h (range 5.1–17.9 h) with T50, T90, and T95 of 7.4 h, 11.1, and 12.6 h, respectively. AST for Enterobacteriacae was finished in 7.2 ± 1.7 h (range 5.1–17.8 h) with T50, T90, and T 95 values of 6.2, 8.7, and 9.6 h. The results for *P. aeruginosa* were available in 12.1 ± 1.9 h (range 6.6–17.8 h) with T50, T90, and T95 values of 12.1, 14.6, and 25.4 h. AST for *S. aureus* was performed in 6.9 ± 1 h, with T50, T90, T95 of 6.7, 7.4, 7.9 h. The mean time to result for CNS was 9.5 ± 2.3 h (range 6.6 h−9.5 h). T50, T90, and T95 were 8.2, 11.4, and 12.9 h. Enterococci were tested in 9 ± 1.9 h (range 5.8–8.9 h), with T50, T90, and T95 of 8.8, 10.4, and 12.6 h, respectively.
Conclusion: The mean time to result for all 3134 bacterial isolates tested was 8.1 ± 2.3 h 50% of the results were ready in 7.4 h. Especially with regard to *S. aureus* 90% of the isolates were tested in one workday in 7.4 h. For Enterobacteriaceae 50% of the isolates were available in 6.2 h and 90% in 8.7 h. With a late shift nearly 70–80% of the results for Gram-positive cocci and Enterobacteriaceae could be reported to the clinical ward on the same workday.

P761 Evaluation of three automated systems: BD Phoenix system vs. Vitek (BioMérieux) and MicroScan WalkAway-96 (Dade-Behring)

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Objectives: Compare the identification profiles (ID) and susceptibility testing results (AST) of Phoenix System (BD Diagnostic Systems, USA) (PHX) with Vitek system version 701 (BioMérieux, France) (Vk) and MicroScan WalkAway-96 system (Dade-Behring MicroScan, USA) (WA). PHX system was also evaluated for general easiness of use in a clinical laboratory with a heavy workflow.
Methods: One hundred and thirty-one clinical strains (51 Gram-negative bacilli (GNB) and 80 Gram-positive cocci (GPC) (29 *Staphylococcus*, 23 *Enterococcus*, 28 *Streptococcus*) isolated in laboratory of University Hospital were tested simultaneously with three equipments. Five type strains were used as quality control, according the recommendations of NCCLS. ID profiles and AST result obtained in the 3 systems were compared. Discrepant results were retested; continuing discrepancy regarding ID was confirmed by Crystal identification system (BD Diagnostic Systems, Sparks, Maryland, USA) and API system BioMérieux and by Etest regarding AST. Categorical agreement (CA) was determined for the antimicrobials to the three systems. Technical performance and the expert system were evaluated.
Results: ID profiles results were similar between the different equipments: agreement between PHX and Vk was 99.26% at the genus level and 87.5% at the species level; agreement between PHX and WA was at 98.7% Genus level and 97.56%. Species level. PHX system didn't require special reagents for the reading of results as well as additional testing while Vk and WA required testing in 6.7 and 1.4% of cases. 2460 AST combinations were evaluated in the three systems. CA rate was 99.2% between PHX and Vk and 99.3% between PHX and WA. WA was the single system that could perform AST for *S. pneumoniae*, just by a visual evaluation. Results regarding detection of extended-spectrum beta-lactamase production (ESBL) in Gram-negative organisms and of methicillin resistant *S. aureus* (MRSA) were similar in the three different systems.
Conclusions: All systems showed to be adequate showing high levels of agreement. PHX presents the possibility of integration on the EpiCenter, a microbiology data management system that can integrate data from other equipment, state-of-art software components, an expert system and data validation logic, without increasing costs. PHX represent an excellent automated tool, easy to use for clinical laboratory personal in large hospitals.
Acknowlegments: Luis Ribeiro, Inês Moreira, Olívia Pinto e Lúcia Ricardo.

P762 Correlation between broth microdilution and *E*-test methods in the MIC determination of colistin in clinical *Acinetobacter baumannii* strains

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Objectives: To evaluate the correlation between MICs of colistin determined by reference broth microdilution and *E*-test methods in clinical *A. baumannii* isolates.
Methods: A total of 101 strains were studied; these isolates were randomly chosen, including 14 strains from a nosocomial outbreak of colistin-resistant *A. baumannii* infections. Broth microdilution: MHIIBCA was used as growth medium; the bacterial inoculum was 6 log10 cfu/mL and concentrations among 0.015 and 128 μg/mL were proved; the MIC was read after 24 h of incubation at 37°C. *E*-test: viable colonies from an overnight agar plate were used and the inoculum was adjusted to McFarland 0.5; manufacturer instructions were followed in the inoculation and application of *E*-test strips; MH agar plates were incubated 24 h at 37°C and the MIC was read at the point of intersection between the inhibition ellipse edge and *E*-test strip. Both experiments were assayed in parallel. *Escherichia coli* ATCC 25922 was used as quality control. MIC breakpoints of colistin were ≤2 and ≥4 μg/mL to designate susceptible and resistant strains, respectively (MENSURA 2000); an *E*-test MIC of 3 μg/mL was rounded up to 4 μg/mL (nearest higher two-fold dilution). Very major (VM) errors were considered when *E*-test showed susceptibility, being resistant by reference method; major (M) errors were considered when *E*-test showed resistance, being susceptible by reference method. Frequency of interpretative errors (VM and M-error classification), Pearson correlation coefficient (r), concordance (±2 dilutions) between methods, and usefulness of *E*-test (sensitivity and specificity) in the detection of resistance were evaluated.
Results: 86% was susceptible and 14% resistant to colistin by broth microdilution. There was one VM error out of 14 strains resistant to colistin, and no M error was found. Significant positive correlation was observed between both methods, although it was very poor ($r = 0.61$). Concordance was also very poor, being below 80% (46%). In the detection of colistin-resistant A. baumannii, the sensitivity and specificity of *E*-test method was 93 and 100%, respectively, with a positive and negative predictive values of 100 and 99%, respectively.
Conclusion: The *E*-test was an useful method in the detection of colistin-resistant *A. baumannii* strains, showing high sensitivity and specificity. However, because its poor concordance, to know the exact MIC it is necessary the use of broth microdilution method.

P763 Ability of Spanish laboratories using automatic susceptibility testing devices to detect and report ESBL-producing Enterobacteriaceae. Results of a multicenter proficiency study

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Objective: To determine the proficiency of Spanish microbiology laboratories in detecting extended spectrum β-lactamases producing Enterobacteriaceae.
Methods: Six Enterobacteriaceae isolates harbouring ESBL were sent to 52 Spanish clinical microbiology laboratories to routinely study their antimicrobial susceptibility. Forty-six laboratories used automatic system (19 MicroScan, 13 Wider, 12 Vitek, and 2 Sensititre) with NCCLS interpretive criteria, but 15 with corrections following the Spanish Antibiogram Committee (MENSURA Group). In addition, 27 laboratories (59%) used the double disk synergy ($n=24$) and/or specific *E*-test ($n=5$) as ESBL confirmatory tests.
Results: The highest rate of ESBL recognition was obtained with an SHV-5 producing *Klebsiella pneumoniae* (74%), a TEM-27 producing *Escherichia coli* (72%), a CTX-M-9 producing *E. coli* (63%), and an SHV-5 producing *K. pneumoniae* lacking both OmpK35 and OmpK36 porins (43.4%). ESBLs were less frequently recognized in two isogenic CTX-M *Enterobacter cloacae* strains, one of them hyperproducing the AmpC enzyme (34.6% and 9.6%). All laboratories using MENSURA interpretations detected at least one ESBL producing *E. cloacae* strain. VITEK users had the highest ability to recognize ESBL producing *E. coli* and *K. pneumoniae*, but lower in ESBL producing *E. cloacae* strains compared with MicroScan and Wider users.
Conclusions: MENSURA criteria favoured the recognition of ESBL phenotypes. ESBLs were recognized to a higher degree in *E. coli* and *K. pneumoniae* strains than in *E. cloacae* strains, due to the lack of NCCLS recommendations for screening and confirmation of ESBL in *Enterobater* spp. and to difficulties to discriminate between the ESBL and the AmpC hyperproduction phenotype in this genus. The CTX-M-ESBL phenotype was less frequently detected than TEM- or SHV-ESBL phenotype due to the lack of cefotaxime-clavulanate combination tests in automatic susceptibility testing systems.

P764 Isolation, characterization and antimicrobial susceptibility testing of *Salmonella* species recovered from cases of septicaemia in an endemic area

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Objectives: (a) Isolation and identification of *Salmonella* species from blood culture specimens of patients of septicaemia. (b) Perform the antimicrobial susceptibility testing using Kirby Bauer method. (c) Perform the agar dilution Minimum Inhibitory Concentration (MIC) testing for Cirofloxacin, Cephotaxime and Cefipime. (d) Detection of Extended Spectrum Beta Lactamase (ESBL) production by these isolates.
Material and methods: One hundred and seventy-eight isolates of *Salmonella* species were recovered from blood culture specimens received over a 21-month period. These were identified using biochemical tests and serotyping with specific antisera. The isolates were subjected to antimicrobial susceptibility screening by Kirby Bauer method for the following agents: Trimethoprim/Sulfamethoxazole, Nalidixic acid, Ciprofloxacin, Crhotaxime, Ceftriaxone, Ampicillin and Cefpirome. The MIC for Ciprofloxacin (178 isolates), Cephotaxime (100 isolates) and Cefepime (100 isolates) was done by the agar dilution method. Extended Spectrum Beta Lactamase production was detected using the Double Disc Screening test using Cephotaxime, Ceftriaxone and Cefoperazone along with Augmentin.
Results: The *Salmonella* species were mostly recovered from the community. Only one strain of *Salmonella senftenberg* isolated from a nursery patient was a hospital isolate. The most common species was *Salmonella typhi* followed by *Salmonella paratyphi* A. One strain each of *Salmonella senftenberg* and *Salmonella typhimurium* were also isolated from neonatal septicaemias. On Disc diffusion testing the *Salmonella typhi* isolates were found to be sensitive to Ciprofloxacin, Cephotaxime, Ceftriaxone and Cefpirome. Nalidixic acid resistance was observed in over 20% of the isolates. The MIC to Ciprofloxacin by agar dilution showed resistance in 3/178 (1.6%) *Salmonella*, MIC to Cephotaxime showed resistance in 2/100 (2%) strains and intermediate sensitivity in 1/100 (1%) and MIC to Cefepime showed resistance in 1/100 (1%) strain.
Conclusions: Resistance to quinolones, third generation cephalosporins and fourth generation cephalosporins is emerging amongst *Salmonella* species. These findings would have important implications in the clinical management of community acquired salmonella infections.

P765 Study to investigate the effect of media depth on the performance of control strains by BSAC methodology

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Objectives: The study was designed to confirm that the depth of media suggested by the BSAC of between 3.5 and 4.5 mm was ideal for the performance of control strains by BSAC methodology.
Methods: The ATCC and NCTC control *P. aeruginosa*, *H. influenzae*, *S. aureus*, *E. coli*, *S. pneumoniae*, and *E. faecalis* strains suggested for use with the BSAC standardized disc testing method were studied. Antibiotics including b-lactam, quinolone, macrolide and glycopeptide antibiotic discs (Oxoid) were tested on Iso-Sensitest agar (Oxoid) (supplemented where necessary) poured to a depth of 2, 2.5, 3, 3.5, 4, 4.5 mm. Plates were stored at 4–8°C and plates were tested on four separate occasions day 1, 5, 10, and 14. Zones were measured by an automated zone reader (Aura, Oxoid).
Data analysis: For the test performance to be acceptable BSAC recommend that 95% of zone diameters for the control strains should be within the published acceptable limits. Each antibiotic/control combination was analyzed to see if it met this criteria.
Results: Table 1 shows the results for discs that did not fulfil the criteria for depths between 3.5 and 4.5 mm. Irrespective of organism/antibiotic combination. When the percentage of tests within the acceptable limits was combined 57, 74, 83, 83, 93, and 91% of tests were within the acceptable limits on media depths of 2, 2.5, 3, 3.5, 4, and 4.5 mm, respectively.

Table 1

		%of tests within the acceptable zone diameter limits					
Control	Disc (µg)	2 mm	2.5 mm	3 mm	3.5 mm	4 mm	4.5 mm
ATCC 25922 *E. coli*	TMP 2.5	0	0	20	3	43	65
ATCC49247 *H. influenzae*	AMC 3	100	100	100	83	48	15
ATCC 49619 *S. neumoniae*	RD 5	45	45	50	48	33	45

Conclusion: The study confirms that the depth of media should be within 3.5–4.5 mm for the ideal performance of the BSAC standardized disc-testing method.

P766 Disk diffusion method for determining susceptibility of *Candida* spp. to voriconazole

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Objective: The purpose was to evaluate the disk diffusion test for determining suceptibility of *Candida* spp. to voriconazole (V) in comparison with the reference method (NCCLS M27-A2 document).
Material and methods: We studied 203 *Candida* spp. (168 *C. albicans*, 26 *C. glabrata*, 9 *C. tropicalis*) isolated from clinical specimens. Susceptibility test was carried out by the agar diffusion method using disk with 1 µg of V (Beckton Dickinson) on Yeast Nitrogen Base (YNB, Difco) plus 1.5% agar. The MICs obtained by the reference broth microdilution method (NCCLS M27-A2 document) (MDB) were defined as the lowest concentration of V that inhibited the 50% of growth. Both methods were read after 24 and 48 h of incubation at 35°C.
Results: At 24 h, 201 strains (96.5%) had MICs values minor or equal to 2 µg/mL, showing 188 (93,5%) of these an inhibition zone diammeter major or equal to 18 mm. Only two *C. albicans* strains (0.98%) showed MICs values major to 2 µg/mL to V by MDB but none of these strains had inhibition zone diammeter minor to 18 mm by disk diffusion method. At 48 h, 144 strains (71%) had MICs values minor or equal 2 µg/mL, showing 106 of these (3.6%) had an inhibition zone diammeter major or equal to 18 mm. Fifty-nine strains

(57 *C. albicans*, 2 *C. glabrata*) (29%) had high MICs values (major to 2 μg/mL) by MDB (13 were resistant and 10 were sensitive dose dependent to fluconazole), 8 of these (6 *C. albicans*, 2 *C. glabrata*) (13.5%) had inhibition zone diammeter minor to 18 mm.

Conclusions:

1. Better correlation between disk diffusion and the reference brtoh microdilution method at 24 h/24 h than at 48 h/48 h for strains with low MICs values to voriconazole.
2. Reading of the inhibition zone diammeter became easier at 48 h than at 24 h.
3. The disk diffusion is a method easier to perform and easier to read than the reference broth microdilution method and is more available to clinical laboratories.
4. More studies are necessary with strains with high MIC values to voriconazole.

P767 Detection of extended spectrum beta-lactamase among *Acinetobacter baumanii* strains

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Objective: To investigate extended spectrum beta-lactamase (ESBL) frequency amongst *Acinetobacter* species isolated from nosocomial infections in a university hospital.

Methods: Hundred nonduplicate *Acinetobacter* strains isolated from clinical specimens were enrolled in the study. Double disk synergy (DDS) was used to detect ESBL frequency. Thirty-microgram ceftazidime, cefepime, aztreonam and ceftriaxone (Oxoid) disks were placed 25 mm (center-to-center) from a 20/10 microgram amoxicillin-clavulunate disk for the conventional DDS test. A clear extension of the edge of inhibition zone of any of the antibiotics towards the disk containing clavulanate disk was interpreted as positive for ESBL production. The test was repeated for the strains by decreasing the distance between the disks to 15 mm.

Results: All strains were identified as *Acinetobacter baumanii* by BBL Crystal (Becton-Dickinson). The strains studied were isolated from tracheal aspiration (39%), blood (31%), wound (14%), urine (9%), catheter (6%), cerebrospinal fluid (1%). No ESBL producing strain was detected when strains tested 25 mm, however by decreasing this distance to 15 mm, 55 of the 100 isolates were demonstrated to be ESBL producers.

Conclusion: *Acinetobacter baumanii* is an important pathogen in nosocomial infections. For this microorganism there is no defined appropriate method for detecting ESBL production. Several studies used DDS method by applying 25 mm distance between the disks and some others 15 mm. Two steps of our study had different results that may affect the clinicians' antibiotic therapy decisions. We concluded that further studies are required to improve detection method.

P768 Rapid Light Cycler PCR for detection of methicillin-resistant *Staphylococcus aureus*

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for an increasing number of serious nosocomial and community-acquired infections. MRSA are resistant to all penicillins, including semisynthetic penicillinase-resistant congeners, penems, carbapenems, and cephalosporins. The basis of this resistance is conferred by an additional gene mecA, encoding the penicillin-binding protein, PBP-2a, which is absent in methicillin susceptible strains.

Methods: We developed a research kit for rapid real time PCR on the Light Cycler Instrument (Roche) to identify the mecA resistance gene. For detection of mecA *S. aureus* colonies are lysed and the DNA is subjected to real time PCR using sequence specific primers and Hyb-probes. PCR products are confirmed by melting point analysis.

Results: The whole procedure, including fully automated DNA preparation, PCR and data interpretation is done within one day. Sensitivity experiments revealed a detection limit of 5–10 genome equivalents.

Conclusion: Thus, the MRSA kit provides a gold standard assay for fast, reliable and sequence specific detection of methicillin resistance in *Staphylococcus aureus* or Coagulase negative Staphylococci in research samples.

Pharmacodynamics

P769 Serum bactericidal activity of penicillin/sulbactam, piperacillin/sulbactam and piperacillin/tazobactam against *Staphylococcus aureus* and *Moraxella catarrhalis*

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Objectives: Conventional in vitro susceptibility tests like MIC and MBC measure the inhibitory activity of antimicrobial agents under standard laboratory conditions only. The serum bacteridical activity (SBA) includes resorption and metabolization of the drug and thus delivers additional pharmacokinetic information. The aim of the study was to compare the SBA of the newly registered narrow-spectrum β-lactam/β-lactamase combination penicillin/sulbactam (Pen/Sul) with the well established broad-spectrum combinations piperacillin/sulbactam (Pip/Sul) and piperacillin/tazobactam (Pip/Taz).

Methods: The study was performed according to the NCCLS guideline. Penicillin G (10 Mega)/sulbactam (1 g), piperacillin (4 g)/sulbactam (1 g) and piperacillin (4 g)/tazobactam (0.5 g) were administered intravenously to six healthy volunteers. Serum concentrations were measured with HPLC. The SBAs against 20 strains of *Staphylococcus aureus* and 10 strains of *Moraxella catarrhalis* were determined 1 and 3 h after drug administration. MICs were determined with the agardilution test. All strains selected were producing β-lactamase and thus were resistant to penicillin.

Results: The MICs of Pen/Sul, Pip/Sul and Pip/Taz for *S. aureus* and M. catarrhalis were $\leq$0.5 and $\leq$0.0015 mg/L, respectively. With *S. aureus*, the geometric means of the bactericidal titres at 1 and 3 h were: 23 and 4 for Pen/Sul, 21 and 3 for Pip/Sul, 21 and 5 for Pip/Taz, respectively. With *M. catarrhalis*, the geometric means of the bactericidal titres at 1 and 3 h were: 630 and 52 for Pen/Sul, 6208 and 1552 for Pip/Sul, 6208 and 676 for Pip/Taz, respectively.

Conclusions: With both *S. aureus* and *M. catarrhalis*, all β-lactam/β-lactamase combinations tested had peak SBAs beyond eight, which is considered to correlate well with clinical cure and bacterial eradication. Against *S. aureus*, which is – beside anaerobes – the main target of Pen/Sul, the narrow-spectrum combination Pen/Sul was as efficacious as the broad-spectrum combinations Pip/Sul and Pip/Taz. With M. catarrhalis, Pip/Sul and Pip/Taz had higher SBAs than Pen/Sul, although the latter also proved an excellent activity.

P770 The effect of protein binding on the activity of Faropenem against *S. aureus*

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Objectives: To predict the efficacy of antibiotics many authors use pharmacological indices which should refer to the nonprotein bound (free) fraction of a drug, as only the free fraction of the antibiotic is active. However, this hypothesis has not yet been proven. To examine this hypothesis, we performed killing curves of *S. aureus* with Faropenem (F), a new oral penem with a protein binding of 94%.

Methods: The kill kinetics of *S. aureus* (MIC 0.125 mg/L) were performed in a batch culture with increasing constant concentrations. The area above the killing curves (AAC) was calculated. Negative values indicate growth of the bacteria whereas positive values indicate bacterial reduction. Furthermore, we determined killing curves in a pharmacological in vitro model over 24 h. The simulated kinetic refers to a 300-mg oral dose of F (tmax 0.5 h, Cmax 11.8 mg/L) and was carried out with and without the addition of 40 g/L

human serum albumin. Additionally, a calculated dosing scheme (6% of 300 mg, t_{max} 0.5 h, C_{max} 0.708 mg/L) resembling the free fraction was simulated.

Results: Figure 1 shows the antibacterial effect vs. concentration of F in a batch culture. The curves demonstrate the effect of F: (i) no albumin is added; (ii) 40 g/L A is added; (iii) 6% of the drug concentration is added. The addition of albumin leads to a decreased antibacterial effect. However, this effect is higher compared with the effect of the free fraction. This tendency could also be seen in the in vitro model. The simulation of a 300 mg dose without albumin results in a reduction of cells of two orders of magnitude and a regrowth after 8 h. The addition of albumin leads to a higher initial reduction of cells but a regrowth already after 6 h. A dosing with only 6% of 300 mg (free fraction) results in a faster and higher initial reduction of cells (2.3 orders of magnitude) than the 300 mg dose without albumin, but the bacteria regrow already after 4 h.

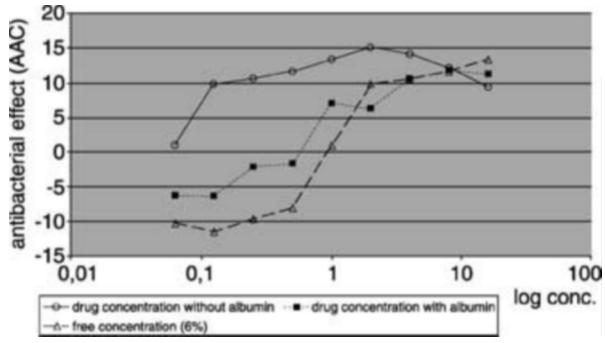

Figure 1 Effect of Faropenem against *S. aureus*.

Conclusion: The hypothesis that only the free fraction of an antibiotic is active cannot be proven. The binding of F to albumin results in a sort of depot. At a concentration of 8 mg/L (total concentration) the effect of protein binding becomes negligible. The stronger reduction of cells of *S. aureus* with lower dosing of F might be explained by an 'Eagle Effect'. However, more data of different strains and antibiotics are needed to see if the results are generalizable.

P771 Under-dosing with teicoplanin remains a problem in the UK

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Objectives: Glycopeptide antibiotics have been the treatment of choice in severe Gram-positive infection for many years, with teicoplanin extensively used in both the UK and much of Europe. Soon after the introduction of teicoplanin, concerns were raised about under dosing and the importance of a loading dose. Although it is not appropriate to monitor teicoplanin concentrations in all patients, there is good agreement between pre-dose levels and outcome. Current recommendations are that pre-dose levels should be >10 mg/L in most serious infections, >20 mg/L for serious staphylococcal infections, but <60 mg/L to avoid potential toxicity. In this study, we report a 9 year review of teicoplanin concentrations.

Methods: A retrospective analysis was conducted of teicoplanin concentrations in samples referred to our laboratory for assay between 1994 and 2002. All samples were assayed by fluorescence polarization immunoassay (Opus Diagnostics Inc).

Results: Over the study period 12 085 requests, from 5720 patients and 258 hospitals across the UK were received. Although the pre-dose concentrations increased slightly over the study period (mean values for the first sample from each patient increasing from 14.7 to 17.8 mg/L), the proportion of patients with acceptable concentrations did not change significantly ($P > 0.05$) over the study period. Overall, pre-dose concentrations were below 10 mg/L (subtherapeutic) in 21.6% of patients, between 10 and 20 mg/L (probably subtherapeutic) in 40.2% and above 60 mg/L (potentially toxic) in only 1.6%. The percentages varying from 23.0, 40.3 and 0.0% (1994) to 17.5, 41.7 and 1.8% (2002), respectively.

Conclusions: Overall, <40% of patients had teicoplanin concentrations appropriate for the treatment of serious staphylococcal infection. In most cases, concentrations were too low, with <2% of patients experiencing concentrations that were considered too high. Although the reasons for assay referral were not known in most cases, initial loading dose recommendations are independent of physiological condition and adequate levels should be achieved in all patients. We conclude that despite recommendations highlighting the importance of adequate loading and maintenance dosing, a significant proportion of patients receiving teicoplanin still have potentially subtherapeutic levels. This situation appears to have changed little over the past 9 years and highlights the continuing importance of therapeutic monitoring for teicoplanin.

P772 The pharmacodynamics of pharmacokinetically enhanced co-amoxiclav and co-amoxiclav 7 : 1 standard formulation on penicillin-resistant *Streptococcus pneumoniae*

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Objectives: Penicillin resistance amongst *Streptococcus pneumoniae* (Sp) remains a cause of therapeutic concern in many countries. Pharmacokinetically enhanced Co-amoxiclav (PeCo-A) produces a greater $T >$ MIC than the standard formulation and hence may have activity against Sp strains with raised Co-A MICs. In this study we used an in vitro pharmacokinetic model to assess the antibacterial effect (ABE) of PeCo-A and amoxicillin: clavulanate 7 : 1 (Co-A 7 : 1) standard formulation against Sp with Co-A MIC greater than or equal to 3 mg/L.

Methods: An open dilutional in vitro model was used to simulate the total serum concentrations of amoxicillin and clavulanic acid associated with oral dosing of PeCo-A and Co-A 7 : 1. The pharmacokinetic parameters modelled were:- PeCo-A Cmax 17.1 mg/L, T_{max} 1.5 h T½ 01.25 h, Co-A 7 : 1 C_{max} 11.6 mg/L, T_{max} 1.6 h & T½ 01.25 h. Six strains of Sp were used with Co-A MICs of 3, 4, 5, 6 (one strain each) and 8 mg/L (two strains). The inoculum used was 10e + 6/7 cfu/mL and each experiment was performed at least in triplicate over 24 h. ABE was measured by log reduction in viable count at 24 h (delta 24, log cfu/mL), time to reduce the viable count by 99.9% of the inoculum (T99.9, h) and the area-under-the-bacterial-kill-curve (AUBKC, log cfu/mLh).

Results: The ABE of the two dose simulations is shown in Table 1.

Table 1

Strain MIC (mg/L)	PeCo-A Δ24	PeCo-A T99.9	PeCo-A AUBKC	Co-A 7 : 1 Δ24	Co-A 7 : 1 T99.9	Co-A 7 : 1 AUBKC
3	−4.0	2	9	−4.2	6	19
4	−4.1	6	16	−4.4	6	23
5	−4.1	6	16	−0.2	7	51
6	−4.1	2	15	−2.2	19	44
8	−3.1	6	20	+2.2	>24	115
8	−4.0	12	22	−3.4	24	41

The $T >$ MIC for 80% maximum effect in these experiments was 41–46%. The $T >$ MIC PeCo-A was 39–61% and Co-A 7 : 1 20–41%.

Conclusion: PeCo-A resulted in a >4 log reduction in viable counts with 5/6 strains, Co-A 7 : 1 only produced a >4 log reduction in the two strains with MIC less than or equal to 4 mg/L. PeCo-A is a more active formulation than Co-A 7 : 1 especially with Sp MIC greater than or equal to 5 mg/L. This is inkeeping with the greater $T >$ MIC with PeCo-A of 39–51% compared with Co-A 7 : 1 of 20–28% with these four strains.

P773 Effect of human serum on the bactericidal activity of faropenem against representative strains of *S. pneumoniae, S. aureus* and capsulate *H. influenzae*

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Objectives: Faropenem daloxate is a highly protein bound oral beta-lactam with in vitro potency against a range of respiratory and skin and soft tissue pathogens. Therefore it is important to establish the effect of human serum on the antibacterial action of faropenem against *H. influenzae*, *S. pneumoniae* and *S. aureus*.

Methods: All three strains used had faropenem MICs of 0.12–0.25 mg/L. A time-kill method was used to test all combinations of 0, 2.5, 5 and 10 mg/L faropenem with 0, 25, 50 or 75% serum. An inoculum of 10e6 cfu/mL was used and samples were taken every 40 min for viable counting up to 6 h. All experiments were performed in triplicate. The bactericidal activity of faropenem was calculated as the difference in area-under-the bacterial kill curve (AUBKC); log cfu/μL/min) between curves with human serum alone and those with serum plus faropenem.
Results: Control experiments (no faropenem) indicated serum had bactericidal activity against H. influenzae; 25 and 50% serum promoted the growth of *S. pneumoniae.* The area-between-the-curves summarizing the activity of faropenem are shown below. The smaller the area, the poorer the activity (Table 1).

Table 1

Faropenum (mg/L)		Human serum (%)	Area-between-curves (log cfu/mL/min) Hi	Sp	Sa
2.5	+	0	839	793	781
	+	25	1059	962	832
	+	50	554	941	908
	+	75	97	790	746
5.0	+	0	936	855	799
	+	25	1172	1021	854
	+	50	706	970	887
	+	75	152	778	730
10.0	+	0	1008	815	854
	+	25	1351	1024	899
	+	50	740	929	906
	+	75	199	789	777

Conclusion: Human serum markedly reduced the activity of faropenem against *H. influenzae* but not against *S. pneumoniae* and *S. aureus*. The effect of protein binding on the antibacterial effect for any antibiotic may vary with bacterial species.

P774 The impact of bacterial inoculum on the antibacterial effect of moxifloxacin studied in an in vitro model of infection

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Objectives: Little is known about the effect that inoculum may have on the antibacterial effect of fluoroquinolones (FQ) in PK in vitro models. In particular inoculum may have an effect on the magnitude of the AUC/MIC required to reach the required antibacterial effect (ABE) endpoint. We studied this issue using *E. coli* at an inoculum of 10e6 cfu/mL and 10e8 cfu/mL and a range of moxifloxacin dose simulations.
Methods: An open dilutional PK in vitro model was used to simulate the serum concentration associated with moxifloxacin at doses of 6.25, 12.5, 25, 50, 100, 200, 400 and 800 mg 24 h for 2 days. Experiments were performed in triplicate and *E. coli* (MIC 0.06 mg/L at inocula of 10e6 and 10e8 cfu/mL) used to assess ABE. ABE was measured by reduction in viable counts at 24 h (delta 24) and 48 h (delta 48) plus the area-under-the-bacterial-kill curve (AUBKC) at 24 h (AUBKC24) and 48 h (AUBKC48). A sigmoid E_{max} curve was fitted to define the dose–ABE relationship.
Results: The relationship between dose and delta 24 was well described by a sigmoid E_{max} model ($r^2 = 0.90$, 10e8 cfu/mL inoculum; $r^2 = 0.86$ 10e6 cfu/mL inoculum). The EC50 dose for 10e8 cfu/mL was 23 mg (CI 17–29) and 10e6 cfu/mL 30 mg (CI 23–40). The dose for 90% maximum effect was 43.7 mg 10e8 inoculum and 53.7 mg 10e6 inoculum. Using AUBKC24 as the ABE the EC50 (10e8 inoculum) was 20 (16–25) and EC (10e6 inoculum) 26 (20–35). Using delta 48 and AUBKC48 did not alter significantly the dose–ABE relationship.
Conclusion: Bacterial inoculum has little impact on the dose–ABE relationship for moxifloxacin vs. *E. coli*.

P775 The antibacterial effect of moxifloxacin against three anaerobic species studied in an in vitro pharmacokinetic model of infection

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Objectives: Moxifloxacin (moxi) has been shown to have in vitro potency against a range of anaerobic species, this combined with its pharmacokinetic (PK) properties would indicate likely anti anaerobic activity in pharmacodynamic models. We used an in vitro PK model to explore the activity of 400 mg/24 h moxifloxacin against *B. fragilis* (B frag) *C. perfringens* (C perf) and Gram positive anaerobic cocci (GPAC).
Methods: An open dilution PK model was used to simulate the free drug concentrations associated with moxifloxacin 400 mg/day (C_{max} 1.6 mg/L, AUC 18.1 mg/L, $T_{½}$ 10.5 h). Experiments were performed at least in triplicate with an inoculum of 10e + 6/7 cfu/mL. Three strains were used with typical moxi MIC values: B frag, moxi MIC 0.25 mg/L, C perf moxi MIC 0.25 mg/L, GPAC MIC 0.12 mg/L. Antibacterial effect (ABE) was measured by change in viable count at 12, 24 and 48 (delta 12, delta 24, delta 48, h) time to kill 99.9% inoculum (T99.9, h) and area-under-the-bacterial-kill curve (AUBKC, mg/L/h).
Results: The in vitro PK model was able to sustain the growth of all three species over 24 h with a 1–2 log increase in viable count over 24 h when no moxi was added. The ABE of a single dose of moxi are shown. With all strains a >2 log drop in viable count was observed at 24 h. Second drug exposures of the three strains resulted in at least >3 log reduction in count at 48 h.
Conclusions: Moxi has significant activity against B frag, C perf and GPAC (MIC less than or equal to 0.25 mg/L) in this in vitro PK model of infection (Table 1).

Table 1

	B frag MIC 1.0	B frag MIC 0.25	C perf	GPAC
Δ12	0.0 ± 0.1	−1.4 ± 0.2	−3.1 ± 0.1	−1.8 ± 0.3
Δ24	+0.1 ± 0.1	−2.4 ± 0.2	−3.2 ± 0.2	−2.4 ± 0.1
T99.9	>24	>24	12	>24
AUBKC 24	95.9 ± 1.0	63.4 ± 3.0	32.6 ± 2.4	57.3 ± 3.7

P776 The pharmacodynamics of oral once a day extended release compared with twice daily standard formulation dosing ciprofloxacin using an in vitro pharmacokinetic model

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Objectives: Cmax/MIC and AUC/MIC are the pharmacodynamic (PD) parameters associated with successful clinical and microbiological outcomes for fluoroquinolones. ODXR CIP 1 g allows a higher C_{max} and once a day dosing compared with CIP 0.5 g BDSF. In this study we simulated total serum concentrations of ciprofloxacin associated with 1 g oral administration as ODXR or 0.5 g BDSF and measured the antibacterial effect measures (ABE) for a range of common pathogens.
Methods: The following isolates were utilized; *E. coli* (EC) (CIP MICs 2, 0.5, 0.03 mg/L), *P. aeruginosa* (PA) (1.5, 0.9 mg/L), *S. aureus* (SA) (2, 0.12 mg/L) at an inoculum of 10e8 cfu/mL. A dilutional in vitro PK model was used to simulate the dosing regimens over 24 h. All experiments were performed in triplicate. The pharmacokinetic parameters modelled were BDSF dosing C_{max} 1.7 mg/L, T_{max} 1 h and ODXR dosing 2.6 mg/L, 3 h for both simulations AUC = 24.2 mg/L and T1/2 = 230min. Antibacterial effect was determined by reduction in viable count at 6, 12, and 24 h; slope and AUBKC 0–24 h. C_{max}/MIC ranged from 117 to 0.9, AUC/MIC from 808 to 12. Statistical analysis was performed using the Sign Test which tests the null

hypothesis that ODXR is equivalent to BDSF based on the direction of differences between the datasets, and the Students *t*-test.
Results: Strains with an AUC/MIC < 125 showed significantly greater killing than strains with AUC/MIC > 125. Analysis of the ABE using the Sign Test showed equivalence between the two regimens. No statistical differences were observed.
Conclusion: Similar antibacterial effects were observed with both dosing regimens. Our data suggests that oral ODXR ciprofloxacin (Ciprofloxacin XR) is equivalent to BDSF dosing (Table 1).

Table 1

Strain MIC mg/L	ODXR Δ24	ODXR S	ODXR AUBKC	BDSF Δ24	BDSF S	BDSF AUBKC
EC 2 mg/L	−0.2	0.21	135	−0.1	−0.17	123.4
0.5	−2.4	−0.15	99.1	−2.4	−0.06	112.4
0.03	−3.1	−0.51	65.4	−5.8	−0.56	33.9
PA 1.5	−0.42	−0.35	106.0	−0.9	−0.82	97.1
0.9	−1.3	−0.49	77.7	−4.2	−0.82	64.5
SA 0.5	−1.4	−0.06	126.0	−3.6	−0.01	124.6
0.12	−1.4	−0.47	86.4	−3.6	−0.37	95.6

P777 Antibiotic tolerance in enterococcus strains

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Objectives: Vancomycin tolerance was investigated in the various clinical specimens. Tolerance can be defined as the ability of bacteria to grow in the presence of high concentrations of antimicrobics, so that the killing action of the drug is avoid but the MIC (minimal inhibitory concentration) remains the same. We investigated vancomycin tolerance in the *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from different clinical specimens.
Methods: Vancomycin was obtained from Sigma Chemical Co. We studied with the total of 100 Enterococcus strains. 56 and 44 of Enterococcus were idendified as *Enterococcus feacalis* and *Enterococcus faecium*, respectively. To determine MICs and MBCs (minimal bactericidal concentration), we inoculated strains from an overnight agar culture to the MHB (Müller-Hinton broth) and shaken for 4–6 h at 37°C to obtain a logarithmic phase culture. The inoculum used, was controlled by performing a colony count for each test.
We used MBC: MIC determinations to study tolerance to vancomycin. Vancomycin tolerance was indicated by MBC:MIC ratio ≤ 32.
Results: We identified Enterococcus strains. 56 and 44 of the Enterococcus strains were identified as *Enterococcus faecium* and *Enterococcus faecalis*, respectively. 31 *E. faecium* and 52 *E. faecalis* were found as susceptible to vancomycin and these susceptible strains were included in this study. The MICs of susceptible strains ranged from ≤1–4 μg/mL. The MBCs ranged >512 μg/mL. A tolerance was detected in 52 *E. faecalis* and 31 *E. faecium* strain. Standard *E. faecalis* 21913 strain also showed tolerance by the MBC:MIC ratio. We defined the tolerant strains with the MBC:MIC ratio ≤ 32. The MBC: MIC ratios of 82 Enterococcus strains were greater than 32. We found a 100% percent tolerance in susceptible Enterococcus strains.
Conclusions: One of the hypothese for tolerance is that, tolerant cells fail to mobilize or create the autolysins needed for enlargement and division. Our data suggests that tolerance may compromise glycopeptide theraphy of serious Enterococcus infections. To add an aminoglycoside to the glycopeptide theraphy unless MBCs are unavailable can be usefull in effective treatment of Enterococcus infections.

P778 In vitro interaction of terbinafine in combination with fluconazole against *Candida albicans* isolates

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Fluconazole (FLZ) is commonly used to threat systemic candidal infections. However it is only fungistatic. Furthermore the isolation of FLZ-resistant *C. albicans* is occuring more frequent and resistance to one azole is usually associated with cross-resistance to other azoles. As the allylamine antimicotic terbinafine (TRB) represents a separate class of antifungals, a combination of TRB with other classes of drugs might be a therapeutic option. In the present study, the in vitro activity of TRB in combination with FLZ were investigated against 31 clinical *Candida albicans* strains. All the strains were isolated from deep mycosis suspected patients' materials (sputum, bronchoalveolar lavage, urine). 2% glucose supplemented RPMI 1640 medium buffered with 0.165 M mops were used. Drug interactions were assessed by a checkerboard microdilution method that adhered to the recommendations of the NCCLS. The final antifungal concentrations (micrograms per milliliters) ranged from 0.125 to 8 for TRB, from 0.125 to 64 for FLZ. MIC endpoints were determined in combination at which the turbidity in the well was less than or equal that in the control growth. Drug interactions were classified as synergistic, additive or antagonistic on the basis of the fractional inhibitory concentration index. The MIC ranges and the MICs (micrograms per milliliters) required to inhibit 50 and 90% of the test isolates (MIC50s and MIC90s, respectively) of TRB were 0.25 to higher than 8 and 8 to higher than 64; of FLZ were 0.25 to higher than 64 and 16–64. The combination of these two antifungals were additive for 18 (58%) and synergistic for 13 (42%) isolates. Antagonism was not observed. These findings are encouraging however, in vitro correlation should be investigated in controlled clinical studies.

P779 Bolus injection vs. 3 h infusion of meropenem in normal volunteers

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Over the last decade, several investigators attempted to establish the most appropriate administration techniques to optimize bectericidal activity of the parenteral antibiotics for the treatment of infections. For beta-lactams, it is generally accepted that the bactericidal effect of these agents is determined by the time that the serum concentrations of antibiotics remains above the MIC ($T > MIC$) for a pathogen. Therefore, continuous infusion would be a mode of administration to maintain such serum concentrations. Meropenem, a beta-lactam antibiotic, is unstable when stored at room temperature in a tropical country (32–37°C) for 8 h. We proposed that 3 h infusion every 8 h would be the appropriate mode of administration of meropenem. Thus, the objective of this study was to compare the $T > MIC$ of meropenem administered by 3 h infusion and bolus injection regimens. The study was three-way crossover study with 1 week wash out period in 12 normal volunteers. Each subject received a single dose of meropenem in three regimens: (i) bolus injection of 1 g-meropenem; (ii) 3 h infusion of 1 g-meropenem; and (iii) 3 h infusion of 0.5 g-meropenem. The meropenem pharmacokinetic studies were carried out in three regimens for 8 h. According to the pharmacokinetic simulations with MIC for the pathogens, the results were shown in Table 1. In conclusion, 3 h infusion of 1 g meropenem would be a mode of administration that can maintain serum drug concentrations above the MIC for most pathogens.

Table 1

	1 g bolus injection			1 g 3 h infusion			0.5 g 3 h infusion		
	4 μg/mL	2 μg/mL	1 μg/mL	4 μg/mL	2 μg/mL	1 μg/mL	4 μg/mL	2 μg/mL	1 μg/mL
% of $T > MIC$	42.50 ± 6.20	54.38 ± 7.64	67.04 ± 8.47	59.27 ± 7.34	71.97 ± 8.63	86.07 ± 9.41	47.27 ± 5.34	69.36 ± 6.60	71.44 ± 8.45

Ertapenem

P780 In vitro activities of Ertapenem and other agents against clinical isolates of anaerobes

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Objectives: Ertapenem, a once-a-day parenteral beta-lactam that was licensed in Europe in April 2002, can be used as a single agent to treat several types of community-acquired and mixed infections. Objectives of this study were to determine the in vitro activity of ertapenem against anaerobes isolated from patients enrolled in ertapenem clinical trials, and compare it with the activities of other agents commonly used to treat the types of infections studied.
Methods: Three double-blind, multicenter studies—complicated intra-abdominal (IAI), complicated skin/skin structure (SI), and pelvic (PI) infection—were conducted worldwide in adults. Patients were randomized to receive ertapenem or piperacillin-tazobactam (PT). Appropriate specimens were cultured for aerobes and anaerobes. Anaerobes were tested using the NCCLS agar dilution method for susceptibility to ertapenem, PT, ceftriaxone (CRO), ampicillin-sulbactam (AS), cefoxitin (FOX), clindamycin (CL), ticarcillin-clavulanate (TC), chloramphenicol (CHL) and metronidazole (MTZ) by agar dilution following NCCLS guidelines.
Results: One thousand seven hundred and eighty-nine anaerobes were studied, including 641 (36%) *Bacteroides fragilis* group, 283 (16%) peptostreptococci, and 227 (13%) *Clostridium* spp. The most common pathogens were *B. fragilis* group in patients with IAI, and peptostreptococci in those with SI or PI. Overall, ertapenem, CHL, PT, AS, and MTZ had excellent antianaerobic activity; CRO was the least active. The rank order of activity against all anaerobes was: ertapenem−98%, CHL−98%, PT−97%, AS−97%, MTZ−96%, TC−92%, FOX−83%, CL−82%, CRO−68%. Only 1% of all anaerobes were ertapenem-resistant, predominantly *Bilophila wadsworthia*. Isolates most often resistant to CHL were *Clostridium* spp.; peptostreptococci, anaerobic Gram-positive bacilli, and *B. ureolyticus*/Bilophila group were most often resistant to MTZ. The most active agents against *B. fragilis* group were MTZ, CHL, and ertapenem. Only 1% of 210 *B. fragilis* strains were resistant to ertapenem; none of 431 other *B. fragilis* group pathogens were ertapenem-resistant. Resistance to ertapenem among Gram-positive anaerobes was rare.
Conclusions: Ertapenem had excellent in vitro activity against anaerobes in this study, including *B. fragilis* and other members of the *B. fragilis* group, recovered from patients with IAI, SI, or PI. Ertapenem was more active against the anaerobes reported here than PT, AS, MTZ, TC, FOX, CL, and CRO.

P781 Ertapenem once a day is highly effective for treatment of generalized peritonitis

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Objectives: Ertapenem is a once-a-day parenteral beta-lactam that can be used as a single agent for treatment of several community-acquired and mixed infections. In a large trial comparing ertapenem 1 g once a day and piperacillin-tazobactam (PT) 3.375 g Q6H for treatment of complicated intra-abdominal infection (IAI), cure rates were 87% (176/203) for ertapenem and 81% (157/193) for PT. One marker of severe IAI is generalized peritonitis. Objectives of this posthoc analysis were to examine the demographics of patients (pts) with and without generalized peritonitis and assess the efficacy of ertapenem vs. PT for treatment of IAI with generalized peritonitis.
Methods: In a double-blind international trial, randomization was stratified by primary site of infection and APACHE II score. The primary efficacy analysis was clinical and microbiological response in microbiologically evaluable (micro eval) pts 4–6 week post-therapy (test of cure [TOC]). Infectious process was classified as generalized peritonitis, single or multiple abscess, or localized disease; for this analysis the latter three were combined as other.
Results: 185 of 623 (30%) treated pts had generalized peritonitis. Compared with pts without generalized peritonitis, those with generalized peritonitis were older (median age, 47 vs. 40.5 year), had slightly higher APACHE II scores (median, 8 vs. 6), and had infections at sites other than appendix (65% vs. 36%). Of pts with generalized peritonitis, 113 (61%) were micro eval, and among them, a higher proportion in the ertapenem group were male (73% vs. 53%), Caucasian (60% vs. 45%), ≥65 year (23% vs. 15%), had appendiceal infection (50% vs. 42%), and had polymicrobial infection (92% vs. 83%), and a lower proportion had postoperative infection (7% vs. 15%). The most common pathogens were *E. coli* and *B. fragilis* group. Median days of therapy were seven for ertapenem and eight for PT. Ertapenem and PT cure rates in micro eval pts were 90% (54/60) and 81% (43/53), respectively, at completion of therapy and 83% (50/60) and 74% (39/53) at TOC. In the microbiologic modified intent-to-treat pts, clinical cure rates were 74% (58/78) for ertapenem and 70% (48/69) for PT. Among those who failed therapy, persistence of a pathogen was documented in two patients in the ertapenem group and three in the PT group.
Conclusion: In this analysis of IAI with generalized peritonitis, which is considered a marker of severe disease, ertapenem 1 g once a day was highly effective therapy.

P782 Ertapenem for treatment of CAP caused by Gram-negative enteric pathogens

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Objective: Ertapenem is a once-a-day parenteral beta-lactam that can be used to treat serious CAP. In the combined analysis of two trials comparing ertapenem and ceftriaxone (CRO) for treatment of serious CAP, efficacy was 92% for both ertapenem and CRO. GN-CAP generally is considered among the more serious infections. Objectives of this analysis were to assess the: (1) demographics and disease characteristics of patients with GN-CAP and CAP due to other pathogens, and (2) efficacy of ertapenem and CRO for treatment of GN-CAP.
Methods: Two prospective, double-blind, randomized studies of CAP in adults, comparing ertapenem, 1 g once a day, with CRO, 1 g once a day, were conducted worldwide.
Randomization was stratified based on Pneumonia Severity Index (PSI less than or equal to three or >3) and age (less than or equal to 65 or > 65). Switch to oral amoxicillin-clavulanate (or other active agent) was allowed after three or more days of parenteral therapy and clinical improvement. Sputum was collected for culture and susceptibility testing of pathogens. The primary efficacy variable was clinical response in clinically evaluable patients 7–14 days after completion of all (parenteral plus optional oral) therapy (test of cure [TOC]).
Results: A pathogen was identified in 402 (47%) of the 857 treated patients; 44 (11%) had Enterobacteriaceae. No Enterobacteriaceae was resistant to ertapenem; five (11%) were resistant to CRO. Compared with the 358 patients with other pathogens, those with GN-CAP were older, and a lower proportion were bacteremic. Of those with GN-CAP, 38 (86%) were clinically evaluable; the proportion of patients with underlying chronic obstructive lung disease, heart failure, and/or diabetes was similar in both treatment groups. Among evaluable patients with GN-CAP, median days of parenteral/total therapy were 4.5/14 for ertapenem and 7/13.5 for CRO, and 85% in the ertapenem group and 72% in the CRO group received oral therapy. Cure rates for ertapenem vs. CRO, respectively, in evaluable patients with GN-CAP were: 95% (19/20) vs. 89% (16/18) at TOC. Cure rates in the modified intent-to-treat population were 91% (21/23) for ertapenem and 90% (19/21) for CRO.

P783 Comparative in vitro activities of Ertapenem against fastidious aerobic bacteria from European patients

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Objective: Ertapenem, a once-a-day parenteral beta-lactam, was licensed in Europe in April 2002 for treatment of several community-acquired and mixed infections. In large clinical trials, it was shown to be highly effective treatment for intra-abdominal, skin/skin structure, urinary tract, and pelvic infections, and community-acquired pneumonia (CAP). The purpose of this study was to assess the in vitro activity of ertapenem against clinical isolates of fastidious bacteria from European patients prior to its clinical use and to compare it to piperacillin-tazobactam and ceftriaxone, the comparators used in the clinical trials.
Methods: In 2001, fastidious bacteria recovered from clinical specimens were tested in 13 centers (11 European countries) by using Etest strips. Interpreta-

tion of MIC values was based on NCCLS breakpoints. Isolates included the common respiratory pathogens – *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, and *Moraxella catarrhalis*, and *Neisseria gonorrhoeae*. In addition to study antimicrobials, susceptibility to penicillin also was evaluated in isolates of *S. pneumoniae*.

Results: A total of 184 *S. pneumoniae* (138 penicillin-susceptible [PSSP], 32 penicillin-intermediate [PISP], 14 penicillin-resistant [PRSP]), 188 *H. influenzae*, 140 *H. parainfluenzae*, 140 *M. catarrhalis* and 55 *N. gonorrhoeae* were tested. The ertapenem MIC range/MIC90 value (mcg/mL) were: PSSP, 0.002–0.125/0.02; PISP, 0.008–1/0.25; PRSP, 0.25–1/0.75; *H. influenzae*, 0.004–0.38/0.12, *H. parainfluenzae*, 0.003–0.15/0.06; *M. catarrhalis*, 0.002–1.5/0.03; N. gonorrhoeae, 0.002–0.125/0.02. For these same isolates, respectively, MIC90 values were 0.3, 1, 3, 0.06, 0.5, 0.05, and 0.02 for piperacillin-tazobactam; and 0.06, 0.25, 32, 0.01, 0.02, 0.5, and 0.02 for ceftriaxone. Based on NCCLS breakpoints, all PSSP, PISP, PRSP, *H. influenzae*, and *H. parainfluenzae* were ertapenem susceptible.

Conclusions: Ertapenem had excellent in vitro activity against these isolates of *S. pneumoniae*, *H. influenzae*, *H. parainfluenzae*, *M. catarrhalis*, and *N. gonorrhoeae* recovered from European patients. The data for the respiratory pathogens plus results of the CAP trials suggest that ertapenem may be an appropriate choice for empiric treatment of many patients with CAP.

P784 Comparative in vitro activities of Ertapenem against aerobic and anaerobic bacteria from Europe

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Objective: Ertapenem, a once-a-day parenteral beta-lactam licensed in Europe in April 2002, has been shown to be highly effective for treatment of complicated intra-abdominal, complicated skin/skin structure, complicated urinary tract, and pelvic infections and community-acquired pneumonia. The purpose of this study was to assess the in vitro activity of ertapenem in Europe prior to its clinical use and to compare it to piperacillin-tazobactam, ceftriaxone and, for urine isolates, norfloxacin.

Methods: In 2001, 13 centers (11 countries) in Europe tested a wide range of Gram-positive and Gram-negative nonfastidious aerobes and anaerobes isolated from clinical specimens by broth microdilution, following NCCLS guidelines. Bacteria generally considered contaminants were excluded from analysis.

Results: Overall 3475 isolates were included in the study. Against all Enterobacteriaceae ($n = 2260$), ertapenem was the most active agent tested; susceptibility rates were 99% for ertapenem, 95% for ceftriaxone, and 92% for piperacillin-tazobactam. For the subset of Enterobacteriaceae from urine ($n = 591$) susceptibility rates were: ertapenem, 99%; ceftriaxone, 94%; piperacillin-tazobactam, 93%; and norfloxacin, 91%. Less than 1% of Enterobacteriaceae were ertapenem-resistant; most of these were from Austria and Belgium. Ertapenem also was the most active agent against the 357 anaerobes: 99% were ertapenem-susceptible vs. 93% for piperacillin-tazobactam, and 48% for ceftriaxone. All agents had excellent activity against methicillin-susceptible *Staphylococus aureus* (MSSA, $n = 198$), *Streptococcus pyogenes* ($n = 169$), and *Streptococcus agalactiae* ($n = 133$), but ertapenem was the most potent, based on MIC90 values. Piperacillin-tazobactam was the only agent active against *Pseudomonas aeruginosa* ($n = 200$), Acinetobacter ($n = 187$), and *Enterococcus faecalis* ($n = 199$).

Conclusions: Ertapenem had excellent in vitro activity against Enterobacteriaceae, anaerobes, MSSA, *S. pyogenes*, and *S. agalactiae* in this study, and was more potent than piperacillin-tazobactam, ceftriaxone and, for urine isolates, norfloxacin. Resistance to ertapenem was not detected in MSSA, *S. pyogenes*, and *S. agalactiae* and was rare (< 1%) in Enterobacteriaceae and anaerobes.

P785 Serious community-acquired pneumonia in patients with chronic obstructive pulmonary disease: utility of Ertapenem

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Objective: Ertapenem (ETP) is a once-a-day parenteral beta-lactam that can be used to treat adults with serious CAP. CAP in patients with COPD is often more difficult to treat than CAP in patients without underlying lung disease. Objectives of this analysis were to compare: (1) the demographic and disease characteristics of treated patients with and without COPD (2) the outcome of evaluable patients with and without COPD, irrespective of therapy, and (3) the efficacy of ETP and ceftriaxone (CRO) for treatment of CAP in evaluable patients with COPD.

Methods: The efficacy of IV/IM ETP, 1 g once a day, was compared with IV/IM CRO, 1 g once a day, in two prospective, double-blind, studies of CAP conducted worldwide. Randomization was stratified based on Pneumonia Severity Index (PSI ≤ 3 or >3) and age (≤ 65 or >65). Patients could be switched to oral amoxicillin-clavulanate or other effective agent after ≥ 3 days of parenteral therapy and clinical improvement. Sputum and blood were cultured; pathogens were tested for susceptibility to study drugs. The primary efficacy variable was clinical response in clinically evaluable patients 7–14 days after completion of all (parenteral plus optional oral) therapy (test of cure [TOC]).

Results: Two hundred and sixty-four (31%) of the 857 treated patients with CAP had COPD. The proportions of patients who were male, were aged ≥ 65 year, had PSI scores >3, and had *Haemophilus influenzae* isolated at baseline were higher in those with COPD than in those without COPD. *Streptococcus pneumoniae* was the most common pathogen in patients with or without COPD. Among evaluable patients with COPD, treatment groups were similar with respect to age, gender, and PSI score; median days of parenteral/total therapy were 4/11 for ETP and 5/11 for CRO; 83 and 77% of those in the ETP and CRO groups, respectively, were switched to oral therapy. Cure rates in evaluable patients at TOC, for the combined treatment groups, were 90% (187/208) for those with COPD and 93% (424/456) for those without COPD. Favorable response rates for evaluable COPD patients at completion of parenteral therapy and at TOC, respectively, were: ETP, 95% (114/120) and 90% (109/121); CRO, 94% (81/86) and 90% (78/87).

Conclusions: Although treatment of CAP in patients with COPD is often difficult, cure rates were only slightly lower than in those without COPD. In this analysis, ETP 1 g once a day, with an oral switch option, was highly effective for treatment of patients with COPD and serious CAP.

P786 Comparative in vitro activities of Ertapenem against clinical isolates of Enterobacteriaceae

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Objectives: Ertapenem is a once-a-day parenteral beta-lactam that was licensed in Europe in April 2002 for treatment of several community-acquired and mixed infections. Objectives of this study were to determine the in vitro activity of ertapenem against Enterobacteriaceae isolated from patients enrolled in ertapenem clinical trials, and compare it with the activities of other agents commonly used to treat the types of infections studied.

Methods: In seven double-blind, multicenter studies, adults with intra-abdominal (IAI), skin (SI), pelvic (PI), or urinary tract infection (UTI) or community-acquired pneumonia (CAP) were randomized to receive ertapenem or comparator: ceftriaxone (CRO; UTI and CAP) or piperacillin-tazobactam (PT; IAI, SI, and PI). Appropriate specimens were cultured for aerobes and, if indicated, anaerobes. Aerobic bacteria were shipped to Merck Research Laboratories and tested for susceptibility to ertapenem, CRO, PT, ampicillin-sulbactam (AS), and ciprofloxacin (CP) by microtiter dilution following NCCLS guidelines. Enterobacteriaceae are the focus of this analysis.

Results: 1768 Enterobacteriaceae were tested: 1221 *E. coli* (69%), 258 *Klebsiella* (15%), 103 *Proteus* (6%), 96 *Enterobacter* (5%), 61 *Citrobacter* (3%), 15 *S. marcescens* (1%), 14 *M. morganii* (1%). Ertapenem was the most active agent tested (100% susceptible); the MIC was less than or equal to 0.5 μg/mL for all but seven *Enterobacter* (< 1% of all isolates). Of the isolates, 97% were susceptible to CRO, 91% to PT, 91% to CP, 61% to AS. Of the 31 CRO-resistant strains, 100%, 55%, 23%, and 6% were susceptible to ertapenem, CP, PT, and AS, respectively. Of the 53 isolates resistant to PT, 100%, 63%, 55%, and 0% were susceptible to ertapenem, CP, CRO, and AS, respectively. 135 strains were CP-resistant; of these 100%, 91%, 67%, and 21% were susceptible to ertapenem, CRO, PT, and AS, respectively.

Conclusions: Ertapenem was highly active against Enterobacteriaceae recovered from patients with IAI, SI, PI, UTI, or CAP, including isolates resistant to CRO, PT, CP, or AS. Ertapenem was more active against these isolates than CRO, PT, CP, and AS.

P787 Phenotypic patterns of antimicrobial cross-resistance in *Pseudomonas aeruginosa*: data from a multicenter US ICU surveillance study

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Objectives: ISS is an ongoing surveillance study of antimicrobial resistance in Gram-negative bacilli (GNB) isolated from ICU patients. The focus of this analysis is *P. aeruginosa*. Objectives were to (1) examine resistance rates in *P. aeruginosa* over time, and (2) explore phenotypic patterns of cross resistance between antipseudomonal agents. Ertapenem, a non-pseudomonal carbapenem, was included to assess its relation to imipenem.
Methods: Each year, participating laboratories tested 100–200 GNB by microdilution following NCCLS recommendations. Only initial isolates of *P. aeruginosa* were analyzed. NCCLS breakpoints for *P. aeruginosa* were used for antipseudomonal agents but are not defined for ertapenem.
Results: 42–97 US laboratories participated each year from 1995 to 2001. During this time, resistance to most antipseudomonal agents increased slightly (2–5%), but resistance to ciprofloxacin increased from 12 to 30%. Among the 1509 isolates tested in 2001, resistance rates for the most active agents were: amikacin, 5%; piperacillin-tazobactam (P/T), 14%; cefepime, 15%; imipenem, 16%; ceftazidime (CTZ), 17%; tobramycin, 17%. Resistance to the combination of an aminoglycoside plus a beta-lactam was lowest for amikacin + imipenem (1.6%). Examples of phenotypic cross-resistance in 2001 isolates are shown in Table 1.

Table 1

CTZ MIC (μg/mL)	*N*	%Imipenem R	% P/T – R	Ertapenem MIC (μg/mL)	*N*	% Imipenem R
≤1	360	<1	1	≤1	124	<1
2	463	11	3	2	169	0
4	257	17	3	4	203	1
8	94	20	3	8	290	1
16	80	31	26	≥16	723	33
≥32	255	34	62			

R = resistant.

Imipenem resistance rates were 42% for P/T resistant isolates and 35% for isolates resistant to ciprofloxacin. 80% of cefepime-resistant strains were resistant to CTZ.
Conclusions: Resistance of *P. aeruginosa* to imipenem increased with increasing CTZ MICs. This trend was stronger than that seen with increasing ertapenem MICs. In contrast to the rates of cross-resistance between antipseudomonal cephalosporins and between CTZ and P/T (which ranged from 62 to 80%), only one-third of *P. aeruginosa* isolates with an ertapenem MIC of 16 μg/mL or more were imipenem resistant, which was similar to the rates of phenotypic cross-resistance between imipenem and CTZ as well as between imipenem and other classes of antimicrobial agents.

P788 Health-care associated pneumonia acquired outside the ICU: results of a randomized, double-blind study comparing Ertapenem and Cefepime

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Objective: Compare efficacy of ertapenem, a once-a-day parenteral beta-lactam, with cefepime for treatment of health-care associated pneumonia acquired outside an ICU.
Methods: A prospective, double-blind (with sponsor blinding), multicenter study was conducted in adults who had pneumonia with onset 48 h or more after hospitalization (outside an ICU) or in a skilled nursing facility. Randomization was stratified by APACHE II score (≤15 or > 15). Patients were randomized (1 : 1) to intravenous (IV) ertapenem 1 g once a day, or IV cefepime 2 g Q12H. Those in the cefepime group could receive IV metronidazole 500 mg Q12H for suspected aspiration; those in the ertapenem group received matching placebo. Switch to oral ciprofloxacin (or other appropriate oral therapy) was allowed after at least 3 days of IV therapy and well documented improvement. Clinical and microbiologic efficacy were assessed 7–14 days after IV + optional oral therapy (test of cure [TOC]).
Results: In the 303 patients randomized, comorbidities were common and generally evenly distributed between treatment groups: congestive heart failure, 24%; prior cerebrovascular accident, 23%; anemia, 21%; chronic lung disease, 21%. The most common pathogens were Enterobacteriaceae (primarily *K. pneumoniae* and *E. coli*), *S. pneumoniae*, and *S. aureus*. 193 (64%) patients were clinically evaluable, and of these, 24% and 21% in the ertapenem and cefepime groups, respectively, had APACHE II scores > 15. Median duration of total therapy for evaluable patients was 10 days in both treatment groups; 59% of patients in the ertapenem group and 53% in the cefepime group were switched to oral therapy. At TOC, clinical cure rates for clinically evaluable patients were 87% (89/102) for ertapenem and 86% (80/93) for cefepime; bacterial eradication rates were 84% (42/50) and 83% (44/53), respectively, for microbiologically evaluable patients. Clinical cure rates per pathogen for ertapenem and cefepime, respectively, were 93% (13/14) and 87% (13/15) for *S. pneumoniae* and 86% (24/28) and 84% (21/25) for Enterobacteriaceae. The frequency of adverse events in this ill population was generally similar in both treatment groups.
Conclusions: In this study, ertapenem therapy, with an oral switch option, was highly effective for treating adults with health-care associated pneumonia acquired outside an ICU. Ertapenem 1 g once a day was as effective as cefepime 4 g daily (+ optional metronidazole) and had a similar overall safety profile.

New anti-staphylococcal drugs

P791 In vitro activities of a novel cephalosporin, CB-181963 (CAB-175), against methicillin-resistant *Staphylococcus aureus*

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Objectives: CB-181963 (CAB-175) is a novel parental investigational cephalosporin belonging to the Azomethines subclass of cephalosporins that has demonstrated in vitro activity against most Gram-positive and Gram-negative bacteria, as well as methicillin-resistant *Staphylococcus aureus* (MRSA). We examined the inhibitory and bactericidal activity of CB-181963 against recent MRSA clinical isolates.
Methods: Two hundred clinical strains of MRSA was obtained from Detroit Medical Center and William Beaumont Hospital in Michigan between 1999 and 2002. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were performed using NCCLS guidelines. The bactericidal activity of CB-181963 was also evaluated with time-kill experiments using four randomly selected MRSA isolates.
Results: See table.

	MRSA ($n = 200$)		MRSA ($n = 4$)
Antimicrobials	MIC_{90} (Range)	MBC Range	Change in 24-h $\log_{10}$ CFU/mL
CB-181963	4 (0.25–8)	1–8	3.87 ± 0.10
Linezolid	4 (0.25–8)	0.5–64	1.68 ± 0.45
Quinupristin/dalfopristin	1 (0.125–2)	0.25–32	2.95 ± 0.90
Vancomycin	2 (0.25–2)	0.5–4	3.86 ± 0.10

Overall, CB-181963 exhibited MIC profiles that were similar to linezolid. However, MBCs and time-kill studies demonstrated that CB-181963 possessed bactericidal activity against MRSA: CB-181963 = vancomycin > quinupris- > linezolid.
Conclusions: CB-181963 demonstrated significant in vitro bactericidal activities against MRSA isolates. CB-181963 may provide an alternative option for the treatment of MRSA infections within the cephalosporin class. However, further pharmacokinetic/pharmacodynamic and clinical investigations are warranted.

P792 Dose-escalation safety and pharmacokinetics study of a novel cephalosporin, CB-181963 (CAB-175): initial human experience

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Purpose: CB-181963 (CAB-175) is a new cephalosporin from the subclass azomethines with in vitro broad-spectrum cidal activity against both Gram-negative and Gram-positive bacteria, including methicillin-resistant *S. aureus* and glycopeptide intermediately susceptible *S. aureus*. This first study in man assessed the safety and pharmacokinetics of CB-181963 at single doses of 250, 500 and 1000 mg.
Methods: In a dose escalation study, a minimum of seven healthy male volunteers was enrolled into each of three dosing groups and randomized (3 : 1) to receive a single dose of CB-181963 (250, 500 or 1000 mg) or placebo. The treatment regimen was intravenous infusion over 30 min. Plasma and urine samples were collected over 24 h from time of infusion and analyzed to determine the pharmacokinetic profile. Safety and laboratory data (including special markers for monitoring effects on the kidney) were collected over 96 h from time of infusion.
Results: Preliminary results indicate that the most frequent adverse event was headache, occurring in eight of the 22 subjects treated. No clinically significant drug-related changes in laboratory data (including renal markers), ECG parameters, or vital signs were reported. The final safety and pharmacokinetic results will be presented.
Conclusions: Initial safety and pharmacokinetic results from this study indicate that further clinical evaluation of a multiple-dose CB-181963 is warranted.

P793 Microbiological characterization of CB-181963 (CAB-175) a novel anti-MRSA cephalosporin

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Objectives: CB-181963 (formerly CAB-175) is a novel cephalosporin with potent activity against a broad range of bacterial pathogens, including methicillin resistant *Staphylococcus aureus* (MRSA). The in vitro antibacterial potency of CB-181963, including susceptibility and bactericidal activity, was characterized against a range of Gram-positive and Gram-negative pathogens. In addition, the potential of susceptible bacteria to develop resistance was probed.
Methods: Minimum inhibitory concentrations (MIC) were determined by broth microdilution according to NCCLS standards, except all cultures were incubated at 37°C. Bactericidal activity was determined by addition of CB-181963 to exponentially growing cultures at 37°C. At various timepoints, samples were removed and diluted at least 1000-fold prior to plating on drug-free agar to determine bacterial viability. To examine the potential development of resistance, bacteria were grown with sub-MIC levels of CB-181963 in Mueller-Hinton broth. From the highest concentration that supported growth, cultures were diluted 1 : 100 000 into fresh media plus CB-181963 at two-fold dilutions; this process was repeated for 21 days.
Results: MIC50/90 values for CB-181963 and Ceftriaxone are shown in Table 1. CB-181963 demonstrates potent activity against MRSA, coagulase-negative Staphylococcus (including methicillin-resistant strains), *S. pneumoniae* (including macrolide, quinoline, and penicillin resistant isolates), and vancomycin sensitive and resistant *E. faecalis* (VSE, VRE), as well as Gram negative pathogens including beta-lactamase negative *K. pneumoniae*. CB-181963 had no significant activity against *E. faecium* or *P. aeruginosa*. The activity of CB-181963 was not affected by the presence of 4% human serum albumin., suggesting low protein binding. As shown in Table 2, CB-181963 was bactericidal (>3 log decrease) at 24 h against all strains tested, including *E. faecalis*, which is typically tolerant of beta-lactams. CB-181963 MICs increased less than five-fold during serial passage of MSSA, MRSA and *K. pneumoniae* strains, suggesting low potential for development of resistance.
Conclusions: CB-181963 displays potent in vitro activity against MRSA as well as broad spectrum activity against clinically important bacterial pathogens. This compound is a promising candidate for further clinical development for broad spectrum use, as well as for treatment of MRSA.

Table 1

		CB-181963 (CAB-175)			Ceftriaxone		
Strain	*N*	MIC50	MIC90	Range	MIC50	MIC90	Range
MRSA	23	2	2	0.5–2	32	>128	8–>128
Coag.neg.Staph	23	1	4	0.25–16	4	>64	0.5–>64
Vanc S *E. faecalis*	45	0.25	0.5	0.125–1	>256	>256	2–>256
Vanc R *E. faecalis*	16	0.25	1	<0.1–1	>128	>128	4–>128
S. pneumoniae	24	0.25	0.5	<0.008–0.5	0.25	1	<0.008–1
K. pneumoniae	30	0.063	0.13	<0.008–16	0.31	0.13	<0.008–1

Table 2

		Average log kill		
Species	*N*	4 h	6 h	24 h
MSSA	3	4.58	4.98	4.98
MRSA	3	1.95	2.53	3.97
GISA	2	1.73	2.56	4.11
VSEfs	3	1.26	2.13	5.63
VREfs	3	2.05	2.44	4.44

P794 The activity of daptomycin against linezoid-resistant Gram-positive pathogens

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Objectives: Antimicrobial resistance is an increasing problem among Gram-positive pathogens. Vancomycin-resistant eneterococci (VRE) are now widespread and vancomcyin (VAN) resistance has recently emerged in *S. aureus*. The oxazolidinone, linezolid (LIN), was introduced into clinical practice in the last couple of years and already there have been reports of resistance. Daptomycin (DAP) is a lipopeptide antimicrobial with broad-spectrum activity against Gram-positive organisms. We have studied the activity of DAP against clinical and laboratory-derived LIN-resistant Gram-positive pathogens.
Methods: The clinical LIN resistant isolates tested were one *S. epidermidis*, two Vancomycin sensitive enterococci (VSE), one VRE, and one *Streptococcus oralis*. Other strains tested were parental strains of MRSA, VRE, and pneumococci, and their derivatives selected for linezolid resistance. Laboratory selection was performed by serial daily passage in Mueller Hinton (MH) Broth containing increasing concentrations of linezolid for 28 days. Susceptibility was tested by agar dilution MIC performed by NCCLS methodology using MH agar (supplemented with 5% blood for pneumococci). Daptomycin MICs were measured on sensitivity testing medium supplemented with 50 mg/L calcium. The agents tested were DAP, LIN, VAN, and Dalfopristin/Quinupristin (D/Q).
Results: The clinical strains were all susceptible to DAP with MICs between 0.25 and 4 mg/L (proposed breakpoint 4 mg/L). LIN MICs for these strains were 16–32 mg/L, one enterococcus was also resistant to VAN (MIC > 128 mg/L), and one enterococcus was resistant to D/Q (MIC 8 mg/L). The results for laboratory-selected strains are shown in the table. As can be seen, all isolates were susceptible to DAP irrespective to resistance to LIN, VAN or D/Q.

		Geometric mean MIC (range) (mg/L)			
	No of strains	DAP	LIN	VAN	D/Q
MRSA (parents)	8	0.47 (0.25–0.5)	1.9 (1–2)	0.56 (0.25–2)	0.69 (0.5–1)
MRSA (LIN selected)	8	0.56 (0.5–1)	13 (8–16)	1.0 (0.25–2)	1.3 (0.5–2)
S. pneumonia (parents)	9	0.16 (0.12–0.25)	0.92 (0.25-1)	0.47 (0.25–0.5)	0.89 (0.5–1)
S. pneumonia (LIN selected)	12	0.12 (0.06–0.25)	14 (4–64)	0.43 (0.12–0.5)	1.5 (0.5–2)
VRE (praents)	2	* (0.25–0.5)	* (1)	* (>128)	* (8)
VRE (LIN selected)	2	* (0.5–1)	* (32–128)	* (>128)	* (8–16)

Conclusions: Daptomycin maintains activity against Gram-positive pathogens which are resistant to other agents such as linezolid, vancomycin, or dalfopristin/quinupristin.

P795 *S. aureus* wound isolate resistance to new antibiotics

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Objectives: *Staphylococcus aureus* is the most common bacterium cultured from infected wounds. There is particular concern about its resistance to methicillin and vancomycin. Consequently, several new antibiotics have been developed. Linezolid and daptomycin have novel mechanisms of action in bacterial infection. Gallidermin is a lantibiotic produced by *Staphylococcus gallinarium*. The goal of this study was to determine resistance to these antibiotics in *S. aureus* clinical isolates and laboratory strains. We previously demonstrated low resistance to standard antibiotics in the same 64 laboratory and clinical strains and we hypothesized that these strains would not be resistant to the new antibiotics.
Methods: In total, 64 different *S. aureus* strains were investigated. Forty-five strains were isolated from wound cultures, five from central venous catheters of ICU patients, and 14 were control strains, consisting of commonly used laboratory strains. The subject population consisted of patients treated at the University of Tübingen (Germany) hospitals and clinics. Strains were isolated in the course of routine clinical diagnostics. Investigations consisted of PCR at the mec (methicillin resistance) locus and assessment of antibiotic resistance at 5 μg/mL, with the exception of daptomycin and linezolid, which were tested at 2 and 4 μg/mL, respectively.
Results:

Table 1 Antibiotic resistance (percentage of strains showing resistance)

	Controls $n=5$	Wound Isolates $n=45$	ICU Isolates $n=5$
Vancomycin*	0	0	0
Methicillin*	21	18	40
mec gene	43	44	60
Linezolid*	0	0	0
Daptomycin*	0	0	0
Gallidermin	0	7	20
No resistance	64	40	0

$P < 0.05$ by Fisher's exact test.
*$P < 0.05$ as compared to other antibiotics by Cochran's Q-test.

Conclusions: Although there were several MRSA strains, no strain was resistant to vancomycin, linezolid, or daptomycin. No control strains were resistant to gallidermin, although there was gallidermin resistance in wound and ICU isolates. This is not surprising given its potential to be encountered in the environment. These results indicate that linezolid and daptomycin are likely to be effective for MRSA infection. Lantibiotic development may be useful but requires further investigation.

P796 Clinical experience with Linezolid in Greek patients with Gram-positive infections

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Objectives: To gain clinical experience in Greek patients (pts) with Gram-positive infections, treated with Linezolid.
Methods: Patients with Gram-positive infections were studied. The duration of linezolid treatment was min 7 days and max 28 days. The schedule of treatment was twice daily either 600 mg per os or 300 mL of 2 mg/mL solution intravenously (i.v.). The patient had to make at least three visits during the study. One visit at baseline, one at the end of treatment and one follow up visit (approximately 15–28 days after end of treatment).
Results: Twenty adult pts (55% male, 45% female) with mean age of 56.9 years (median 61 year, SD 18.8 year) were studied. All of them were hospitalized for skin/soft tissue (15 pts) or lower respiratory infections (four pts) and one case of osteomyelitis (allergic to methicillin, clindamycin, vancomycin and teicoplanin). Linezolid was given i.v. or orally or switched from i.v. to oral treatment at the discretion of the investigators. Simultaneously, four diabetic pts received ofloxacin 400 mg twice daily for soft tissue infections of the lower extremities and one received rifampicin 900 mg daily for osteomyelitis. Underline conditions were hypertension, diabetes, cardiac failure, asthma, Parkinson's disease, peripheral vein insufficiency, migraine, cirrhosis, renal insufficiency, gastrectomy (gastric carcinoma). Median duration of i.v. treatment was 8 days and of oral treatment 6 days. Median duration of treatment for skin and soft tissue infections was 14.0 days (SD 4.49 days), for pneumonia 9.0 days (SD 0.5 days) and for osteomyelitis 45 days. All of them were cured and none of them discontinued therapy. Adverse events that occurred were generally mild to moderate and resolved during continuous treatment, except of one patient with worsening renal failure. Headache, skin rash, oral candidiasis, fever, anemia, thrombocytopenia, increase of liver enzymes were reported. Forty-five percent of the enrolled patients experienced the reported adverse events.
Conclusion: Linezolid has the potential to be an alternative to vancomycin and may find a major role in the treatment of infections caused by Gram-positive microorganisms and especially by MRSA or other difficult-to-treat pathogens as long as the potential for resistance is minimal. Further clinical investigation will help determine its place in the treatment of these infections.

P797 In vitro activity of daptomycin against Gram-positive uropathogens

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Objectives: The incidence of nosocomial UTIs caused by Gram-positive bacteria, resistant against current antibiotics, e.g. fluoroquinolones, is increasing. Daptomycin, a recently developed antibiotic, active against Gram-positive bacteria should be tested for its antimicrobial activity against uropathogens.
Methods: The antimicrobial activity of daptomycin was tested against pathogens from three different collections: (i) Uropathogens from hospitalized urological patients (1990/91) with complicated and/or hospital-acquired UTIs of the Hospital St. Elisabeth, Straubing. (ii) Uropathogens from a multicenter study (1995/96) on complicated UTI comprising 37 urological centers throughout Germany. (iii) MRSA isolates of patients and staff (1999/2000) within the Hospital St. Elisabeth, Straubing. Genotyping of the latter isolates was performed by pulsed-field-electrophoresis. The minimal inhibition concentrations (MIC) of daptomycin, were determined by an agar (calcium supplemented isotonic sensitivity agar) dilution method using a multipointer with an inoculum of 104 cfu per point.
Results: For all methicillin susceptible *S. aureus* (MSSA) ($n=25$), for methicillin resistant *S. aureus* (MRSA) ($n=49$), for methicillin susceptible coagulase-negative staphylococci (CNS) (MSSE) ($n=129$), for methicillin resistant CNS (MRSE) ($n=33$), for *E. faecalis* ($n=289$), and for *E. faecium* ($n=4$) the MICs ranged up to 2 mg/L indicating that all strains were susceptible to daptomycin.
Conclusion: According to the in vitro activity daptomycin may be considered a promising antibacterial agent for the treatment of complicated UTI caused by Gram-positive uropathogens. Thus, daptomycin should be evaluated in a clinical study.

P798 Vancomycin to linezolid switch in patients with MRSA infection

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Objectives: Linezolid is a new treatment option for resistant Gram-positive cocci infections. Due to its favorable bioavailability it can be used as a follow-up option for IV treatment. We describe the clinical consequences of patients (Pts) with MRSA infections, treated initially with vancomycin (VA) and latter switched to linezolid.
Methods: Retrospective cohort study. Thirty-eight Pts with MRSA infection treated with VA and switched to linezolid, were included. Data were collected on demographics, comorbidities, clinical characteristics, antibiotic treatment, indications for switch, recurrence of MRSA infection, failure of treatment, occurrence of thrombocytopenia, leukopenia, and renal failure. Criteria for the likelihood of these events to be related to linezolid were set a priori.
Results: Pts mean age was 62 years (35–85), 66% were female, 50% were surgical Pts, and 47% were in ICU before the switch. Pts were severely ill as

expressed by Charlson score of 3.7 (range 0–10). The most common comorbidities included cardiovascular disease (71%), renal disease (45%), and diabetes (39%). Indication for VA treatment included: bacteremia or sepsis (40%), soft tissue infections (29%), nosocomial pneumonia, and mediastinitis (8% each). Treatments was switched to linezolid due to subsequent isolation of VA resistant enterococcus (VRE), in 66%; VA associated allergy/adverse effect 10%, VA failure 10%, and in 14% with main aim to switch to oral antibiotics. The switched occurred after a median 7 days of VA treatment (mean 19 days). Linezolid was prescribed for a median 5 days in the hospital (mean 9 days); and 10 patients were discharged on oral linezolid (median 14 days). Microbiological failure (recurrence of MRSA) occurred in only one Pt (with arterial graft infection). Hematological events during linezolid treatment: Thrombocytopenia occurred in three Pts in only one case it was likely related to linezolid and in other it was unlikely related to. Leukopenia occurred in one patient, unlikely to be related to linezolid. New renal failure occurred in one patient, unlikely to be related to linezolid. No drug events, except the one case with thrombocytopenia required discontinuation of linezolid.
Conclusion: Pts with MRSA infection were switched to linezolid mostly due to coinfection with VRE, or to VA related drug effect or failure. The switch appeared to be safe both with respect to recurrence of MRSA infection, and in respect to side-effects.

P799 Comparative in vitro activity of TD-6424, a rapidly bactericidal, concentration-dependent antibiotic with multiple mechanisms of action against Gram-positive bacteria

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Objectives: Unlike other glycopeptides, TD-6424 has been shown to inhibit bacterial lipid synthesis in addition to inhibiting peptidoglycan synthesis. TD-6424 has potent activity against Gram-positive bacteria and exhibits rapid, concentration-dependent bactericidal activity. We compared the in vitro activity of TD-6424 with that of vancomycin, teicoplanin, linezolid, quinupristin/dalfopristin, moxifloxacin and ampicillin, penicillin or oxacillin as appropriate, by the NCCLS/EUCAST and BSAC methods.
Methods: The organisms ($n = 401$) included in the study were patient isolates from St. Thomas' Hospital and were selected to include representatives of the clinically important Gram-positive aerobic species. The isolates were further selected to include, where possible, those known to have specific resistance mechanisms including vancomycin, methicillin and erythromycin resistance. Susceptibility testing was performed by agar dilution methods on Mueller-Hinton agar according to the NCCLS/EUCAST guidelines in comparison to Isosensitest agar according to the BSAC guidelines. Both media were enriched with 5% horse blood for fastidious organisms. The minimum inhibitory concentration was defined as the lowest drug concentration that prevented bacterial growth.
Results: TD-6424 was active against all the Gram-positive species tested and nearly 90% of isolates included in the study had TD-6424 MICs < 1 mg/L. Vancomycin-resistant enterococci and lactobacilli isolates with vancomycin MICs > 64 mg/L had TD-6424 MIC ranges of 0.5–8 and 2–16 mg/L, respectively. There was no evidence of cross-resistance with other comparator drugs. The results for TD-6424 for the two methods were mostly either the same or within one doubling dilution.
Conclusions: The susceptibility breakpoints have yet to be established but it would appear that TD-6424 has superior activity to the other tested glycopeptides and on a weight for weight basis displays comparable or better activity than the other agents tested with activity against Gram-positive bacteria.

P800 Oritavancin demonstrates in vitro activity against susceptible and resistant enterococci collected in 2000–2001

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Objectives: Vancomycin (VAN)-resistant (R) enterococci are commonly encountered pathogens in the hospital setting. As antimicrobial resistance among enterococci, especially *Enterococcus faecium* (EM), continues to increase, the need for new agents capable of evading current resistance mechanisms becomes greater. Oritavancin (ORI) is a semisynthetic glycopeptide antimicrobial that has previously shown potent in vitro activity against enterococci, including VAN R EM and *Enterococcus faecalis* (EF).
Methods: We examined the in vitro activity of ORI, VAN, and comparator agents against 238 EM, 401 EF, and 77 *Enterococcus* spp. (Espp.; non-EM and non-EF) collected from patient specimens at hospital laboratories in 15 European countries. Isolates were selected to include both susceptible (S) and R phenotypes. All isolates were centrally tested using NCCLS broth microdilution methodology, and MICs were interpreted using the 2002 NCCLS published breakpoints.
Results: ORI had an MIC90 of 1 mg/L against all EM and showed consistent activity against VAN S (MIC90, 1 mg/L) and VAN R (MIC90, 2 mg/L) isolates. ORI had an MIC90 of 2 mg/L against all EF; MIC90s were 2 mg/L against VAN S EF and 2 mg/L against VAN R EF. Against Espp., ORI had an MIC90 of 1 mg/L against all isolates and MIC90s of 1 and 2 mg/L against VAN S and VAN R isolates, respectively. Against vanA ($n = 55$ EM, 30 EF) and vanB ($n = 13$ EM, 5 EF) phenotypes, ORI had MIC90s of 2 and 2 mg/L, respectively, against vanA phenotypes and 1 and 2 mg/L, respectively, against vanB phenotypes. ORI showed consistent activity against high-level streptomycin (HLS; 1000 mg/L) R EM ($n = 151$; MIC90, 1 mg/L) and EF ($n = 189$; MIC90, 2 mg/L) and against high-level gentamicin (HLG; 500 mg/L) R EM ($n = 137$; MIC90, 1 mg/L) and EF ($n = 44$; MIC90, 2 mg/L). ORI had an MIC90 of 2 mg/L against 21 pan R EM, defined as R to ampicillin, VAN, HLG, and HLS.
Conclusions: ORI showed consistent activity against EM and EF, including those isolates that were R to multiple antimicrobials. Based on these data, ORI may have potential as a therapy for R enterococcal infections.

P801 Activity of Oritavancin against susceptible and resistant *Staphylococci* collected in Europe during 2000–2001

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Objectives: The burden of antimicrobial resistance (R) among staphylococci is a continuous challenge faced by physicians worldwide. Increases in the prevalence of oxacillin (OX) R among staphylococci are often seen in conjunction with increases in R to other antimicrobials, thereby making the treatment of patients with staphylococcal infections more difficult. Oritavancin (ORI) is a semisynthetic glycopeptide that has shown potent in vitro activity against *Staphylococcus aureus* (SA) and coagulase-negative staphylococci (CNS).
Methods: We tested 701 SA and 303 CNS against ORI, vancomycin (VAN), OX, and comparator agents by NCCLS broth microdilution. Isolates were originally collected during 2000–2001 from patient specimens in 15 European countries and were chosen to include both susceptible (S) and R phenotypes. MICs were interpreted using the 2002 NCCLS published breakpoints.
Results: ORI had an MIC90 of 2 mg/L against all SA and showed identical activity against both OX S ($n = 483$; MIC90, 2 mg/L) and OX R ($n = 218$; MIC90, 2 mg/L) isolates. One SA and one CNS had VAN MICs $= 4$ mg/L; the corresponding ORI MICs were 2 mg/L. Against CNS, ORI showed an MIC90 of 2 mg/L with identical activity against OX S ($n = 140$; MIC90, 2 mg/L) and OX R ($n = 163$; MIC90, 2 mg/L) isolates. Among the isolates tested, 190 SA and 113 CNS were multidrug-resistant (MDR), defined as R to ≥ 3 antimicrobial classes. ORI showed consistent activity against non-MDR and MDR SA (MIC90s, 2 mg/L) and CNS (MIC90s, 2 mg/L), including isolates that were resistant to five antimicrobial classes (OX, ciprofloxacin, erythromycin, gentamicin, trimethoprim-sulfamethoxazole).
Conclusions: ORI showed consistent activity against SA and CNS, irrespective of R to OX or other antimicrobials. ORI is currently in Phase III clinical trials for the treatment of Gram-positive infections and may possess potential as a therapy for R staphylococci.

New antibiotics – quinolones and miscellaneous

P802 Mutational studies with a new fluoroquinolone, DK-507k, against Gram-negative bacteria: comparisons with six fluoroquinolones

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Objectives: The objectives of this study were to evaluate the selection of mutational resistance by a new fluoroquinolone (FQ), DK-507k (DK), against three Gram-negative bacterial species and to compare with ciprofloxacin (CIP), levofloxacin (LEV), gatifloxacin (GAT), moxifloxacin (MOX), sitafloxacin (SIT), and garenoxacin (GAR) In addition, the potency of these FQ against the mutants selected were also compared.
Methods: *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), and *Haemophilus influenzae* (HI) were selected for this study. FQ potency was measured by agar dilution methodology. Mutant selection studies were performed in vitro using two methods. Gradient plate methodology was used to select mutants with subinhibitory concentrations of FQ over seven culture passages. The second method involved selection of single-step (SS) mutants with superinhibitory concentrations of FQ. Changes in susceptibility to the FQ were evaluated with the mutants selected.
Results: In studies with EC, SS mutants were selected with each FQ. SIT, DK and CIP were the most active against these SS mutants with MICs < 0.25 mg/L. Higher levels of resistance were selected with gradient plates. Surprisingly, LEV was most active against these mutants (MIC = 8 mg/L) with other FQ being two- to fourfold less active. In studies with HI, SS mutants were only selected with DK, GAR, GAT, and CIP. All FQ still maintained MICs of <0.12 mg/L against these HI mutants. Although larger increases in MICs were observed using gradient plates, MICs for all FQ against these mutants remained below 1 mg/L. In studies with PA, SS mutants were selected with each FQ. MICs increased up to eightfold, with all mutants remaining susceptible to <4 mg/L of SIT and CIP, and <16 mg/L of DK and LEV. Similar to EC and HI, mutants from gradient plates exhibited much higher levels of resistance, with MICs for all FQ being >16 mg/L.
Conclusions: The potency of DK, CIP and SIT against SS mutants of EC and HI suggest that these drugs may be effective in slowing the emergence of resistance in these bacterial species. With all FQ, mutational to resistance remains a serious threat with PA. Pharmacodynamic and further mutational studies with DK against other Gram-negative pathogens are warranted.

P803 Mutational studies with a new fluoroquinolone, DK-507k, against staphylococci and streptococci: comparisons with 6 fluoroquinolones

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Objectives: The objectives of this study were to evaluate the selection of mutational resistance by a new fluoroquinolone (FQ), DK-507k (DK), from four staphylococci (Sta) and two streptococci (Str), compared with ciprofloxacin (CIP), levofloxacin (LEV), gatifloxacin (GAT), moxifloxacin (MOX), sitafloxacin (SIT), and garenoxacin (GAR) In addition, the potencies of these FQ against the mutants selected were also compared.
Methods: Two *Staphylococcus aureus* and two *S. epidermidis* (one methicillin-susceptible and one resistant strain for each species), and one strain each of *Streptococcus pneumoniae* and *S. pyogenes* were included in this study. FQ potency was measured by agar dilution methodology. Mutant selection studies were performed in vitro using two methods. Gradient plate methodology was used to select mutants using subinhibitory concentrations of FQ over seven culture passages. The second method involved selection of single-step (SS) mutants with superinhibitory concentrations of FQ. Changes in susceptibility to the FQ were evaluated with the mutants selected.
Results: DK, GAR, and SIT were the most potent FQ (MIC = 0.015–0.06 mg/L). Mutants with high-level resistance were readily selected with gradient plate method. Against the Sta mutants, DK and SIT were significantly more potent with MICs of <2 mg/L against most mutants. It was harder to select SS Sta mutants with DK and SIT compared with other FQ. MICs for DK, SIT, GAR, and MOX against all SS mutants were <0.5 mg/L. SS mutants were selected from both Str strains with all FQ. DK, SIT, and GAR were significantly more potent than other FQ against the SS pneumococcal mutants with MICs < 0.5 mg/L. Against the *S. pyogenes* SS mutants, DK and SIT were also significantly more potent with MICs < 0.06 mg/L. DK, SIT, and GAR were the most potent FQ against the mutants selected by gradient plates. However, it was surprising that GAR actually selected mutants with significantly higher levels of resistance than the other FQ.
Conclusions: The Gram-positive activity of DK is a strength of this compound. The potency of DK and lower propensity of DK to select less susceptible Sta mutants is very comparable to that of SIT. These finding suggest these two agents may be effective in slowing the emergence of FQ resistance among Gram-positive bacteria.

P804 Post-antibiotic effect studies with a new fluoroquinolone, DK-507k, and six other fluoroquinolones

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Objectives: The objectives of this study were to evaluate the postantibiotic effects (PAEs) of a new fluoroquinolone (FQ), Dk-507k (DK), against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*, compared with ciprofloxacin (CIP), levofloxacin (LEV), gatifloxacin (GAT), moxifloxacin (MOX), sitafloxacin (SIT), and garenoxacin (GAR).
Methods: All four bacterial species were FQ-susceptible. Log-phase cultures (10^6–10^7 cfu/mL) were exposed to each FQ at a concentration 10× the MIC and incubated at 37°C. As the control, similar cultures were incubated in the absence of FQ. After 1–2 h, dependent upon rate of killing, drug was removed by centrifugation and 2× washing with sterile buffer, and cells were resuspended in fresh 37°C growth media and incubated at 37°C for 5 h. Viable bacterial counts were measured at time of initial inoculation, at the end of drug exposure, after removal of drug and resuspension in fresh media, and at hourly intervals thereafter. PAEs were defined as the time required for FQ-exposed cultures to increase 1 log after drug removal minus the time required for control cultures to increase 1 log after receiving the same centrifugation and washing treatment.
Results: Against *E. coli*, DK, CIP, and MOX exhibited the longest PAEs of 3 h, compared with 2.5 h for SIT, and 1–1.5 h for GAT, LEV, and GAR. Against *P. aeruginosa*, DK, LEV, GAR, GAT, and MOX exhibited PAEs of 1.5–2 h, compared with 0.5–1 h for CIP and SIT. Against *S. aureus*, only CIP exhibited any substantial PAE (2 h). The other FQ either failed to exhibit a PAE or exhibited a short PAE of only 0.5 h. Against *S. pneumoniae*, GAT, GAR, and MOX exhibited the longest PAEs of 3.5–4 h, compared with 2 h for DK and SIT, and 1 h for CIP and LEV.
Conclusions: DK exhibited its longest PAEs against *E. coli* and *S. pneumoniae*. In comparison to other FQ, DK exhibited one of the longest PAEs against the Gram-negatives. DK was similar to most FQ in its lack of PAE against *S. aureus*, and exhibited a moderate PAE against *S. pneumoniae* compared with the most active FQ. In general, the PAEs exhibited by DK were very comparable to those exhibited by other FQ that are dosed once daily. Of interest would be further studies evaluating the PAEs of DK in the presence of subinhibitory concentrations of drug.

P805 Molecular analysis of *Streptococcus pneumoniae* mutants selected by DK-507k, an investigational fluoroquinolone

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Objectives: DK-507k (DK) is an investigational fluoroquinolone (FQ) with potent activity against Gram-positive pathogens. A study was designed to determine the molecular mechanisms involved when less susceptible mutants of *Streptococcus pneumoniae* (SP) were selected by DK and six comparison FQs.
Methods: The six comparison FQs were sitafloxacin (SIT), ciprofloxacin (CIP), levofloxacin (LEV) and moxifloxacin (MOX), gatifloxacin (GAT) and BMS-284756 (BMS). SP strain R6 was used for generation of mutants by seven serial passages on gradient antibiotic plates containing increasing concentrations of drug. Mutants selected during the first, fourth (p4) and seventh (p7) passage were evaluated for changes in quinolone susceptibility by

comparing agar dilution MICs (NCCLS methodology) to those of the parent strain. The QRDR regions of gyrA, gyrB, parC and parE, the ATP binding region (ABR) of parE and the efflux associated gene pmrA of p4 and p7 mutants were amplified by PCR and directly sequenced.
Results: The p4 mutants had MICs corresponding to reduced susceptibility to the selecting drug and also to the comparison drugs tested while the p7 mutants had the highest MICs obtained for all drugs tested in this study. The mutations observed in this study were previously associated with FQ resistance in *S. pneumoniae*. The mutations found in p4 mutants were also present in p7 mutants. The most common mutation was Ser(81)Tyr in gyrA followed by Ser(79)Tyr in parC. Except for the p7 mutants selected by MOX and GAT (which had one mutation), the p7 mutants selected by the other drugs had at least two mutations. The mutation profile of DK-p4 was Ser(81)Tyr in gyrA and Ser(79)Tyr in parC while the mutation profile of DK-p7 was Ser(81)Tyr, and Glu(85)Lys in gyrA and Ser(79)Tyr in parC. Such mutations correlated with a 32-fold (p4) and 128-fold (p7) increase in DK MICs. Only the SIT-p7 mutant had changes in three genes, gyrA, gyrB and parC. No base change was noted in any mutants for parE, pmrA and the ABR of parE.
Conclusions: DK and the comparison FQs exhibited correlation between mutational changes and increases in MIC. DK appears to have the characteristics of a dual target drug that did not show any preference for gyrA or parC as its primary target.

P806 Efficacy and safety of telithromycin in two treatments of acute sinusitis in adults

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Objective: Telithromycin is the first antimicrobial agent of the Ketolide group that is in the market. It has a promising spectrum of activity against respiratory pathogens including *S. pneumoniae* Pen-R strains. Forty-one Greek patients (27 males, age range 19–65 years) were recruited in an international, multicentre, randomized, double-blind study, aiming to evaluate the clinical efficacy and safety of telithromycin in the treatment of acute sinusitis in adults.
Methods: All subjects had acute sinusitis, clinical and radiologically confirmed and underwent transantral needle puncture with aerobic and anaerobic culture performed in the obtained specimens. In 20 subjects, 800 mg of telithromycin once daily was administered for 10 days (group A) and in 21 subjects for 5 days followed by placebo for the remaining days (group B). Criteria of successful treatment were full remission or improvement of the symptoms and signs of acute sinusitis as well as the elimination or improvement of radiological findings.
Results: Ten positive cultures from sinus aspirates revealed *S. pneumoniae* (3), *Streptococcus* spp. (1), *Staphylococcus* CNS (2), *H. influenza* (1), *S. aureus* (3). All strains were sensitive to telithromycin. In 18 subjects of group A and 18 of group B therapy was considered successful. One each subject deteriorated in both groups and one each had clinical and radiological findings that remained unchanged. Mild adverse events were observed in two subjects. No clinically significant laboratory abnormalities or QTc changes were identified.
Conclusion: The 5- and 10-day treatment regimens with telithromycin were equivalent in efficacy with only minor side-effects. Treatment failures were possibly due to domination of anaerobic species in the pathogenesis of the infection as confirmed by the subjects positive response to the administration of additional therapy with antianaerobic activity.

P807 Antimicrobial activity of tigecycline (GAR-936) against multiresistant *Acinetobacter baumannii*

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Objectives: Multiresistant *A. baumannii*, including resistance to carbapenems, is a common cause of severe nosocomial infections. The objective of this study was to determine the in vitro antimicrobial activities of a new glycylcycline, tigecycline, and imipenem against clinical *A. baumannii* isolates.
Methods: Forty-nine strains from bacteremic patients were studied, belonging to the two more frequent clones genotyped by REP-PCR in our hospital, with 22 and 27 strains, respectively. Thirty-seven strains were multiresistant, defined as resistance at least to three antimicrobials: carbapenems, ceftazidime, ciprofloxacin, amikacin, and/or sulbactam. The MICs and MBCs values for each strain were determined by broth microdilution method according to NCCLS 2000. Because there is not an approved standard for considering *A. baumannii* susceptible or resistant to tigecycline, MIC breakpoints used for this agent were those for minocycline: $\leq$4, 8, and 16 μg/mL to designate susceptible, intermediate, or resistant isolates, respectively. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as quality control strains. Bactericidal activity of tigecycline and imipenem was evaluated using time-kill curves using nine strains with different susceptibilities to imipenem (IMP): three IMP-susceptible (S), three IMP-intermediate (I), and three IMP-resistant (R); tigecycline or imipenem concentrations corresponding to the MIC, 2×MIC and 4×MIC for each isolate were tested. Bactericidal activity was defined as $\geq$3 log 10 reduction compared with the initial inoculum.
Results: MIC50/MIC90 of tigecycline and imipenem were 2/2 and 32/128 mg/L, with a range of 1–4 and 1–128 mg/L, respectively. MBC50/MBC90 were 8/>8 and 32/128 mg/L for tigecycline and imipenem, ranging from 2 to >8 mg/L, and from 1 to 128 mg/L, respectively. Percentages of susceptible isolates were 100 and 28% for tigecycline and imipenem, respectively. By time-kill studies, tigecycline was not bactericidal; imipenem was bactericidal against seven strains (3S, 2I, and 2R).
Conclusions: (1) Tigecycline is active against multiresistant *A. baumannii* strains, including those imipenem-resistant. (2) The results of the time-kill studies show that tigecycline is bacteriostatic against these strains.

P808 Effects of S-carboxymethylcysteine on the attachment of *Streptococcus pneumoniae*

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Objectives: Respiratory infections are preceded by colonization of the pharynx by bacteria. Therefore, inhibition of bacterial colonization in the pharynx seems to be a useful strategy to prevent infections. S-carboxymethylcysteine (S-CMC) is perhaps the most promising agent since previous studies have shown that it significantly reduced the number of episodes of acute exacerbations of respiratory infections. The effects of S-CMC on Gram-positive bacteria and bacteria with a well-defined capsule have not been investigated. The present study was designed to examine the effects of S-CMC on the attachment of *S. pneumoniae* to human pharyngeal epithelial cells (HPEC).
Methods: The following strains of *S. pneumoniae* were used in this study: strain SP-95-27 (serotype 9V); strain SP-95-19 (19F); strain SP-96-29 (6B); and strain SP-95-36 (14). All were isolated from respiratory specimens. Assays were done to find out the effects of S-CMC on the attachment and detachment of *S. pneumoniae* to HPEC.
Results: The attachment of strain SP-95-19 to HPEC significantly ($P < 0.05$) decreased following treatment of the cells with S-CMC at a concentration of 100, 10, 1 or 0.1 μg/mL. However, at 0.01 and 0.001 μg/mL, S-CMC had no effect on the attachment. Treatment of HPEC with S-CMC at a concentration of 0.1 μg/mL resulted in a significant ($P < 0.001$) decrease in the attachment of the other three strains of *S. pneumoniae*. For detachment assay, HPEC with the attached *S. pneumoniae* strain SP-95-19 were treated with 100, 10, 1 or 0.1 μg/mL S-CMC. This resulted in a significant decrease in the number of attached bacteria. However, no such decrease was noted with 0.01 and 0.001 μg/mL S-CMC. The detachment assay also showed a significant ($P < 0.05$) decrease in other three strains of *S. pneumoniae*, with 0.1 μg/mL S-CMC. Treatment of strain SP-95-19 with 10 or 1 μg/mL S-CMC significantly decreased the number of bacteria attached to HPEC. However, at concentrations of 0.1 and 0.01 μg/mL, S-CMC had no significant effect on attachment. Treatment of the other three strains of *S. pneumoniae* with 10 μg/mL of S-CMC resulted in a significant ($P < 0.001$) decrease in the number of attached bacteria.
Conclusion: S-CMC can modulate the attachment of *S. pneumoniae* to HPEC by acting both on the cells and bacteria at a concentration, which is achievable in respiratory secretion in vivo. This drug may be potentially useful for the prevention of *S. pneumoniae* infections.

P809 Inhibition of endotoxin-induced cellular activation by polymyxin B-albumin conjugate

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Objective: The endotoxin from Gram-negative bacteria consists of a molecule lipopolysaccharide (LPS), which can be shed by bacteria during antimicrobial

therapy. The resulting syndrome, endotoxic shock, causes organ failure, shock and death. Thus, there is great interst in the development of antiendotoxin agents, which can reverse rather than promote sepsis. We describe here covalent PMB-HAS conjugate, which has antiendotoxin activity.
Methods: Conjugates of PMB with HSA were prepared by EDAC [1-ethy-3-(3-dimethylaminopropyl) carbodiimide]. Double immunodiffusion agar test with immunized rabbits sera was performed. Fresh heparinised blood from normal doners was collected to prepare peripheral blood mononuclear cells. After incubation and washing nonadherant cells, the monocytes were stimulated with LPS and various concentration of PMB-HSA conjugate. Supernatants were collected for measurement of TNF alpha using bioassy with L929 cells and ELISA. Peritoneal macrophages were isolated from C57BL/6 mice, and stimulated with LPS and various concentration of PMB-HSA conjugate. Nitric oxide formation was measured in culture supernatants with the Griess reagent.
Results: Double immunodiffusion agar test showed that purified conjugate contained bound PMB. The PMB bound in the conjugate retained its endotoxin-neutralizing activity compared with that of unbound PMB as evidenced by its dose-dependent inhibition of TNF release from human monocytes (in vitro), and nitric oxide production from murine macrophages, which stimulated by purified *E. coli* O55:B5 LPS.
Discussion: These experiments demonstrated that the PMB-HSA conjugate retains bound yet functional PMB and may be useful for the design of drug for treatment of Gram-negative bacterial sepsis.

P810 Antimicrobial activity of 2,4′-bipyridyl ammonium salts

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Objectives: A series of novel 2,4′-bipyridyl ammonium salts with varying lengths of alkyl chains were synthesized and tested as antimicrobial agents against *Staphylococcus aureus*, *Acinetobacter*, *Escherichia coli*, *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*.
Methods: Chemical synthesis was based on the reaction between 2,4′-bypyridyl and 1-bromoalkane. The structure of the compounds was confirmed by their physico-chemical characteristics, elemental analysis and UV and IR spectra and their purity by TLC antimicrobial activity was measured by inoculating disks with 100 μg of the agents and recording the inhibition of different bacterial strains on Mueller–Hinton agar.
Results: The compounds displayed excellent antimicrobial activity against *S. aureus*. Lesser activity was observed against *Acinetobacter*, *E. coli* and *S. maltophilia*. *P. aeruginosa* was completely resistant to the compounds but the resistance could be overcome by adding membrane permeabilizing agents such as EDTA and sodium citrate. For each species tested the antimicrobial activity varied according to the length of the alkyl chains. The most active compounds had alkyl chains of between 9 and 12 carbons. The parent dipyridyl compound and the compounds with the longest alkyl chains (C14, C16) had no appreciable activity.
Conclusions: The proposed mechanism of action is by interaction with the cell membrane These compounds represent a promising new series of drugs for further testing.

P811 Antirhinovirus activity of new 2-styrylchromones

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Human rhinoviruses (HRVs) constitute the most extensive genus of the Picornaviridae. The more than 100 serotypes of HRVs are the major cause of common cold, an acute infectious disease widespread in developed countries. No licensed effective antiviral is available at the moment for the treatment of common cold. The in vitro antipicornavirus activity of flavonoids, either synthesized by us or from natural sources, is well known. Because of the broad spectrum of activity and of the interesting mechanism of action, we synthesized new flavonoid derivatives (2-styrylchromones) to improve their antiviral effect and to better define structure–activity relationship. The antiviral potency of new compounds has been evaluated against HRV 14 and HRV 1B infection of (Ohio) HeLa cells. HRV 14 and 1B have been selected as representative serotypes for group A and B of HRVs, respectively, as identified by a different susceptibility of all HRVs to a panel of antiviral compounds. The activity has been tested in a plaque reduction assay, starting from the maximum concentration of compound which did not affect cell viability and growth. Results obtained from structure–activity relationship studies suggest that the introduction of a methoxy or hydroxy substituent in third position of the chromone ring enhances the antiviral potency against both serotypes tested; the introduction of a chlorine substituent on the chromosome ring leads to a reduction of activity for both 3-hydroxy- and 3-methoxy-2-styrylchomones; among the 2,4-pentadien-1-ones, the 3-hydroxy substituted compounds result more potent against HRV 1B and less active towards HRV 14 than the corresponding compounds without substituent on the chain. In conclusion, our data indicate that 2-styrylchromones represent a new class of antirhinovirus flavonoids, exhibiting activity against both group A and B of HRVs.

P812 Efficacy of dimerized vs. monomeric magainins against nosocomial bacterial pathogens

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Objectives: Magainin is a cationic peptide derived from the skin of frogs. It is known to have antimicrobial properties due to its ability to bind to bacterial LPS and induce spontaneous pore formation. Previously, we have synthesized native magainin peptides and those containing a cystine residue, which can be induced to form dimers (Dimagainin). Using *Stenotrophomonas maltophilia* as the test organism, we have shown that dimerized magainin is 1000 times more efficient at killing cells than an equivalent concentration of monomeric magainin. The reason for this is that the rate limiting factor of pore formation by magainin is dimerisation of magainin monomers. In this study, we have tested the range of organisms killed by monomeric and dimeric magainins.
Methods: Bacterial kill assays were performed by adding magainin or Dimagainin (1 μm monomer equivalents) to broth cultures (500 cfu/mL) for varying lengths of time, followed by plating serial dilutions of the resultant cell suspension onto nutrient agar and growing overnight at 37°.
Results: Consistently, when magainin was shown to reduce viable count, dimerized magainin reduced the viable count around 100 times more at a given time point. Monomeric magainin (1 μM) was not able to reduce viable counts to zero for any bacterial strain tested. Viable counts were reduced to zero after 30 min incubation of clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* with 0.5 μM dimerized magainin It took 90 min incubation with 0.5 μM dimerized magainin to reduce the viable count to zero of *Salmonella typhimurium*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*. The growth of Gram-positive organisms, such as *Staphylococcus aureus* and coagulase negative streptococci was not inhibited by either monomeric or dimeric magainins, nor was the growth of *Providencia stuartii*.
Conclusions: Probably due to its more rapid killing of bacteria, 0.5 μm dimerized magainin is able to reduce the viable count of many clinically important Gram-negative bacteria to zero, even using starting culture densities of 500 cfu/mL. This adds further evidence that dimerized magainin would make a more efficacious topical antimicrobial agent than the already clinically used monomeric form of the peptide.

P813 Tomatozid – a new perspective antiviral and immunomodulating drug

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This study investigated the antiviral activity of tomatozid (a steroid glycoside extracted from tomato seeds) against human viruses (Picornaviridae, Reoviridae, Herpesviridae) and its influence on the immune system. In vitro antiviral activity was evaluated using an index of neutralization (IN, the difference between the titre of viral replication in the absence and in the presence of tomatozid): IN = Ig (CPE50)/mL (where CPE is the cytopathic effect). The antiviral activity of tomatozid was found to increase simultaneously with its dose. The most efficient concentration was 0.05–0.1%. IN = 3.14–3.7 [lg(CPE50)/mL] for different virus families. For experiments in vivo, we used mice infected with Herpes simplex virus (HSV-1). The IN was 2.56 [lg(LD50)/0.03 mL] (where LD is lethal dose). In the experimental group, 95.8% of mice survived. The mean duration of life was 20.25 days (the period of observation was 21 days). For the reference group the corresponding values were 58.08% and 16.64 days. In a comparative study, the antiviral activity of tomatozid was compared with the activity of a reference interferon sample (Leukinferon, Reaferon, Realderon). The IN was 3.0 [lg(CPE50)/mL] for

Tomatozid and 1.8–2.4 [lg(CPE50)/mL] for interferon. Immunomodulating activity in vitro was estimated using the Lymphocyte Blast Transformation Test. Index of stimulation (IS) of lymphocytes exposed to phytohemagglutinin was IS = 11. Next we added to the test system different concentration of tomatozid. At a concentration of 0.01 μg/mL tomatosid stimulated blast transformation (IS = 18). Concentration of 0.1 μg/mL had no evident effect (IS = 12). High concentration (1.0, 10.0, 100.0 μg/mL) manifested inhibiting action on lymphocyte transformation (IS = 7–0). The experiments in vivo confirmed the immunostimulating action of tomatozid. Histologic study of lymphoid tissue in mice treated with tomatozid showed evident proliferative changes: large lymphoid follicles with developed germinal centers and accumulation of many blast cells. In conclusion, tomatozid has a significant antiviral effect upon RNA- and DNA-containing viruses. Tomatozid proves also immunomodulating activity (low doses-stimulating action, high doses-suppressing action). The antiviral activity degree of tomatozid does not yield to the reference human interferon samples. The interferonogenous effect of tomatozid has been stood out, which would clear up a possible antiviral action mechanism of the tested substance.

Clinical fungal infections and nosocomial infections

P814 A *Fusarium oxysporum* strain isolated from a case of keratitis

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Keratomycosis is a significant worldwide ophthalmic problem. *Fusarium* spp. are common soil saprophytes and plant pathogenes which in humans are mostly associated with superficial mycosis and keratitis. We report a *Fusarium oxysporum* strain isolated from a case of keratitis after corneal injury by a plant matter. A 40-year-old female patient presented with a trauma history in October 2002. Only one specimen obtained by swab was sent to deep mycosis lab due to perforation risk of deteriorated epithelial layer. In direct microscopic examination of the specimen stained with Ehrlich-Ziehl-Neelsen, methylene blue, Giemsa techniques revealed several individual one-celled fungal elements. Specimen was inoculated onto Sabouraud dextrose agar, brain–heart infusion agar, cooked sheep's blood agar. The plates were incubated at 35, 30, 25°C. After 4 days cultures on SDA showed whitish mycelial growth with pale orange colored in the center. The reverse was concentrically brownish, orange and whitish color. The fungus was isolated as a single microorganism from specimen and was transferred onto oat meal agar, potato dextrose agar, potato sucrose agar, Czapek Dox agar, malt extract agar and incubated at 25°C in diffuse daylight for 7–10 days. The isolate produced whitish, floccose colonies 4–5 cm in diameter. The colony reverse of subcultures was pale white on all agar media. The isolate produced numerous one to two celled oblong microconidia in variable size on short and often unbranched conidiophores bearing monophyalides. Macroconidia were aboundant, having 3–5 cells, more or less curved, pointed at both ends. The chlamydospores were mostly globose, hyaline, smooth-walled, terminal or intercalary. The isolate was identified as *F. oxysporum*. Susceptibility testing of the strain was performed against conventional antifungals according to NCCLS M38-P guideliness. MICs (micrograms per milliliter) were found as follows: amphotericin B 16, fluconazole higher than 64, itraconazole higher than 16, ketoconazole 16. Fungal elements could be inoculated into the cornea by traumatic instrument such as a plant matter at the time of injury and then the cornea is susceptible to opportunistic fungal infection. Seasonal variation has been noted in several keratomycosis series in USA, India. Corneal infection by hyphomycetes peaked during late spring and late autumn. *Fusarium* spp. are the predominant fungi causing keratomycosis during those months like in the present case.

P815 Clinical and epidemiologic data of 40 cases with candidal balanitis

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Objectives: The recognition of favorable and epidemiologic factors and clinical features of candidal balanitis.
Methods: During the period, March 1995–August 2002, we have observed 40 patients of age 14–67 years with candidal balanitis. The diagnose was proved by microscopic examination and growing in culture of Sabouraud terrain with chloramphenicol [we consider positive the presence of 15 and more colonies/culture]. We analyzed the frequency according to age groups; favorable factors [unprotected sexual contacts accompanied pathologies; immune suppressive therapies, prepuce status] and clinical features of the disease.
Results: The epidemiologic analyses: (a) Cases related to age group were: 14–20 years 1 case, 21–30 years 11 cases, 31–40 years 16 cases, 41–50 years 6 cases; 61–67 years 3 cases. (b) Favorable factors unprotected occasionally contacts 35 cases; diabetes mellitus 2 cases; long immune suppressive therapy 2 cases; prepuce interest and/or its constriction 20 cases. Clinical analysis: (a) Subjective signs: dysuria 11 cases, pruritis 14 cases; painful glans or prepuce 16 cases; discomfort during coitus 34 cases. (b) Lesion location: glans 15 cases, glans and prepuce 16 cases, glans and balano-prepucial sulcus 3 cases, glans and frenulum 4 cases, glans and urethrael meatus 2 cases. (c) Macroscopic aspects: small painful erythemas 3 cases, diffuse erythema 8 cases, moist papule 3 cases, erosion-ulcer 8 cases, abrasions-rhagades 7 cases, pseudo membranous 11 cases. (d) According to course: acute forms 22 cases, prolonged forms [over 1 month−14 months] 18 cases. (e) Complications: phymosis presence 13 cases and urethra meatitis 2 cases.
Conclusions: The most affected age group of that candidiasis was 21–40 years, 67.5% of the cases; the most frequent favorable factor resulted unprotected sexual contact 87.5% of the cases; more often we met balanoposthitis 40% of the cases and balanitis 37.5% of the cases. We identified six types of macroscopic lesions of the disease.

P816 The influence of fungal colonization in the colon mucosa on the activity of IBD/ulcerative colitis and Crohn's disease

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Background: Three main interactive factors such as: host susceptibility, enteric microflora and mucosal immunity play role in the pathogenesis of IBD. The importance of presence of fungi in digestive tract remains unclear. The aim of study was to: (1) Estimate the frequency of fungal colonization of colon mucosa in patients with ulcerative colitis (UC) and Crohn's disease (CD). (2) Identify the fungal species. (3) Evaluate the differences in the frequency of fungal colonization between groups of patients with the duration of disease, less and more than 5 years. (4) Evaluate the influence of antifungal treatment on the course of IBD. (5) Examine the coexistence of pathogenic bacteria with fungi.
Material and methods: We investigated 84 patients, admitted to the Department of Gastroenterology of University Hospital in Cracow in active phase of UC/64 cases, 27 duration less and 33 more than 5 years/and 20 cases of CD/12 less, 8 more than 5 years of duration/aged from 18 to 72 years. Clinical investigations included the history of disease, colonoscopy with biopsies from changed colon mucosa, brush smears from mucosal surface. Mycologic tests/210 samples/included biopsies from colon mucosa, brush smears, blood and stool samples. Quantitative and qualitative tests for the presence of fungi and bacteria were performed. For evaluation of the activity of disease, histopathology of colon mucosa and the level of CRP in the blood were estimated.
Results: Significant fungal colonization, more than 105CFU/mL was found in 35–78% of UC patients and 15–47% of CD cases with the history of disease less -more than 5 years respectively. In three cases of UC patients and two cases of CD patients, fungal antigen was isolated from the blood. *Candida/C. albicans* was isolated in 92.6% and *C. glabrata* in 7.2% of cases. In 30%—25% of UC–CD patients, respectively, fungal colonization coexisted with such bacteria as: *E. coli*, *Proteus* spp., *Klebsiella* spp. Introduction of antifungal therapy caused clinical remission in all positive cases.
Conclusions: (1) Fungal colonization is one of the factors inducing exacerbation of UC and CD. (2) *C. albicans* was the most important species isolated from patients with IBD. (3) Longer duration of IBD, more than 5 years is a risk factor of significant fungal colonization of the colon. (4) Introduction of antifungal treatment induced clinical remission of IBD in all positive cases.

P817 *Trichosporon asahii* causing fetal septic shock in a patient without neutropenia

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Objective: *Trichosporon asahii* has been reported as a cause of fungemia, especially in patients who have neutropenia and hematologic malignancy. We describe a patient without hematologic malignancy and neutropenia representing septicemia resulting from *Trichosporon fungemia.*
Case report: A 57-year-old woman was performed subtotal hysterectomy, right oopherectomy and omental biopsy. Pathologic examination revealed ovarian serous papillary cystadenocarcinoma. She was then initiated a chemotherapy regimen (carboplatin and paclitaxel) in postoperative period. During and after chemotherapy period, no neutropenia was devoleped. After chemotherapy she was re-operated for left oopherctomy and bilateral pelvic adenectomy. On the second day of this operation she was admitted to intensive care unit (ICU) due to the development of acute respiratory distress syndrome. On ICU day 2, her respiratory failure was progressed and she was intubated and re-operated due to the intra-abdominal hemorrhage and increased intra-abdominal pressure (abdominal compartment syndrome). On day 8, her fever was 38.2°C, culture of blood and endotracheal aspirate samples revealed methicillin-resistant *Staphylococcus aureus* and a chest X-ray revealed bilateral pulmonary infiltration. Teicoplanin was initiated. On day 15, her fever was 39.3°C and she developed septic shock and imipenem was added to the antibiotherapy after blood samples were withdrawn for culture. These samples cultured *T. asahii* within 48 h, imipenem was discontinued and amphotericin B started. Cultures obtained from vascular and abdominal drainage catheters were negative for *T. asahii*. Throughout the patient's hospital stay and during cancer chemotherapy she had never neutropenia. On the 3rd day of antifungal treatment, the patient died. The blood isolate was identified as *T. asahii* morphologically and by the means of Vitek API 20 C biochemical (BioMerieux, France) testing system.
Conclusion: Fungemia due to *Trichosporon* species has been an emerging state in patients with hematologic malignancies and neutropenia. We demonstrate here with this case presentation that *Tasahii* may also cause life threatening sepsis in patients who do not have neutropenia.

P818 Disseminated cryptococcosis in an HIV-negative patient: case report

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A 45-year-old male patient was presented with altered consciousness, somnolence, headache, dizziness, vertigo, nausea, vomiting, obtundation, clumsiness, weight loss. Physical findings; axiller temperature 37°C, pulse rate: 100/minute, blood pressure: 180/100 mmHg. Nuchal rigidity was detected. Laboratory studies: leukocytes13000/mL, hemoglobin 16 g/dL, hematocrit 46%, thrombocyte 146000/mL, sedimentation 10 mm/h, CRP 0.6 mg/dL, HBsAg, anti-HCV, anti-HIV were all negative. Blood chemistry, chest roentgenogram, cranial CT were normal. The cerebrospinal fluid (CSF) was clear, colorless, increased opening pressure, Pandy reaction was positive, leukocytes 128/mL (95% lymphocyte), protein 87 mg/dL, glucose 30 mg/dL, concomitant blood glucose 160 mg/dL Gram and Giemsa stains of CSF showed cells with large, white capsules. India ink preparation and culture of CSF revealed cryptococci. Blood and urine cultures were also positive. Latex particle agglutination antigen titers for *Cryptococcus neoformans*; 1 : 8000 in CSF, 1 : 4000 in blood. Cranial MRI showed nonspecific vasculitic and ischemic lesions without contrast enhancement. Antifungal sensitivity test revealed fluconazole and flucytosin resistance and amphotericin B sensitivity. Non-lipid amphotericin B (amp B) was started. The patient developed left facial paralysis, chemosis in both eyes, papilledema was detected. Two weeks later; plasma urea and creatinine levels elevated by more than 50% (127 and 2.4 mg/dL, respectively). Non-lipid amp B was discontinued and liposomal amp B treatment was planned for 8 weeks. During the fourth week of liposomal amp B, fluconazole (800 mg, i.v.) was added because of slow response and development of incomplete lost in left eye, complete visual loss in right eye. After 8 weeks, oral fluconazole maintenance therapy was started. Further investigation; T-cells subgroups, immunoglobulin levels, C3-C4 levels were normal. RF were negative. Ppd: 10 mm. Thorax CT was normal. Abdominal CT showed a 4.5 × 2.5 cm hypodens adrenal mass. Adrenal MRI T1-T2 images confirmed an iso-hypointens mass. İ V Gd DTPA showed homogen contrast enhancement. Blood levels of adrenal hormones were normal (incidental adrenal adenoma). Four months later; while parenchymal cryptococcomas seen on first MRI enlarged. Neurologic findings improved completely except visual loss. Latex particle agglutination antigen titers of CSF 1:2000, blood 1:1600. No recurrence was observed during controls.

P819 Recurrence of cryptococcal meningoencephalitis in a patient with idiopathic CD4+ T lymphocytopenia

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Objectives: To report a case of relapsed cryptococcal meningoencephalitis in a patient with idiopathic CD4+ T lymphocytopenia. A 75-year-old heterosexual white male was admitted to the hospital because of severe headache, fever, nausea, fatigue and weight loss for 4 months. There was a history of skin and central nervous system cryptococcal infection 6 years ago, then treated with excision of the lesion and 5-flucytosine for a period of 6 weeks. A lumbar puncture was performed and the cerebrospinal fluid (CSF) revealed an opening pressure of 12 cm H_2O, a cell count of 52/mm^3 (NE 18, LY 26, non-recognizable 8), a total protein value of 198 mg/dL and a glucose level of 11 mg/dL. The CSF India ink stain for fungus were negative, but the serum and CSF cryptococcal antigen titers were positive for *Cryptococcus* sp. at 1/128 and 1/512, respectively. In addition, *Cryptococcus neoformans* was isolated from CSF cultures. The total number of patient's WBC was within the normal range, but a persistent lymphopenia (a systemic lymphocyte count of approximately 500/μL) was detected in every examination. In order to complete the investigation of the patient's lymphopenia the immunophenotype of peripheral blood and bone marrow were performed. A persistent low CD4 + cell count of approximately 17/mm^3 was present in the peripheral blood of the patient, combined with normal CD8. The patient had no risk factors for HIV infection and he has been repeatedly tested negative for HIV-1 and HIV-2, as well as by Quantiplex HIV RNA 3 (bDNA) technique. The ANA, ANCA, C3, C4 and ACE tests were also negative. No pathologic findings were detected at the CT scans of the upper and lower abdomen, as well as at the CT and HRCT scans of the thorax. The gastroscopy and colonoscopy examinations were also normal. The bone marrow biopsy revealed a nonspecific slightly hypoplastic bone marrow tissue. The cytology examination of the CSF was negative for the existence of neoplastic cells. The therapeutic regimen followed was intravenous amphotericin B (0.5 mg/kg/day) in combination with flucytosine (150 mg/kg/day divided into 4 dosages) for 2 weeks, followed by per os fluconazole in a dosage of 800 mg/day for 5 days and then 400 mg/day until today.
Conclusions: Cryptococcal meningitis is a life threatening opportunistic infection in patients with idiopathic CD4 lymphocytopenia. The occurrence of relapses suggests that a chronic suppressive therapy is needed.

P820 Severe imported maduromycosis poorly responsive to multiple antifungal treatments: a rare case report treated with liposomal amphotericin B and voriconazole, and literature review

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Introduction: Maduromycosis (M) is a chronic disease usually involving lower limbs. An aspecific onset (papular-nodular lesions, subcutaneous abscess), has a slowly progressive evolution borne by local edema, avascular necrosis, and tendency to fistulization and discharge of typical brown-black granules. Coming from tropical regions, M is rarely observed in nonendemic areas, mostly as an imported disease, so that only anecdotal reports are described. Its correct management relies first on the distinction between a bacterial origin (*Nocardia* and *Actinomyces* spp.),and a fungal one (with >20 different species involved). A 45-year-old male immigrated from Kenya 13 years before, suffered from a severe M involving the left lower limb, already detected in 1987. Multiple complications interested bone, joints, muscle, ligaments, tendons, and soft tissues, leading to foot deformity associated with severe functional deficits, in absence of systemic disease, despite frequent episodes of bacterial superinfection, which contributed to worsen local fibrosis, ischemia, and ulceration. Istopathologic studies showed a granulomatous process containing macrophages and giant multinuclear cells, and confirmed the fungal

etiology (PAS-positive spheric chlamydosporae, arthrosporae, and true hyphae typical of *Madurella mycetomatis*). Notwithstanding multiple courses of amphotericin B, ketoconazole, fluconazole, and itraconazole (often associated with therapy for bacterial complications), a chronic-relapsing evolution continued, and the severe bone-joint damage (documented by X-ray and CT scan studies), required a surgical debridement of necrotic and fibrotic areas, in association with the administration of liposomal amphotericin B, followed by voriconazole.
Conclusion: The emerging of M as an imported disease with a disabling course should focus attention on both diagnostic and especially treatment features. Clinicians and microbiologists should be aware of M, when history and epidemiologic clues may suggest it, although M remains a very infrequent disease at our latitude. Differential diagnosis between bacterial and fungal etiology is the first key step, since its recognition may be delayed by the cumbersome identification of Actinomyces and *Nocardia* spp., and the fungal organisms. Medical therapy of M of fungal origin remains an unresolved problem, due to the unpredictable efficacy of antimycotic therapy. Among newer agents, voriconazole deserves attention for its potential activity against most of fungi causing M.

P821 Colonization and infection with *Candida* species in adult hematology wards

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Objective: Most data on the epidemiology of *Candida* species are from the United States, mainly studying the epidemiology of candidemia. European studies have focused on specific groups of patients such as neonates, patients with malignancies or HIV infections. Data on the epidemiology of *Candida* species in adult hematology unites are scarce. The epidemiology of *Candida* species in our hematology unit has not previously been studied. The aims of the project were to describe trends in the incidence of colonization and infection with candida species in our hematology unit during the study period, to describe the molecular epidemiology of the isolates and to make recommendations on surveillance and prevention of nosocomial candidal infections.
Methods: The study was conducted for 3 months period (from March to May 2002). Isolates from routine surveillance samples, taken from patients on admission, and weekly thereafter during their stay, were included, in addition to any isolates from clinical samples taken at the time of suspected infection. Samples from staff hands and environmental samples from areas of high patient and staff hand contact were taken using transparent adhesive tapes. Identification and antifungal susceptibilities of strains were carried out using standard laboratory methods. A randomly amplified polymorphic DNA method (RAPD) was used for fingerprinting of isolates.
Results: A total of 725 samples were taken from patients during the study period and 112 (15.44%) were positive for Candida species. Of the 167 Candida species isolates recovered from patients during the study period 116 (70%) *C. albicans*, 39 (23%) *C. glabrata*, 8 (5%) *C. tropicalis*, 2 (1%) *C. krusei* 2 (1%) *C. parapsilosis*. Twelve (10%) and 9 (8%) *C. albicans* isolates were resistant to fluconazole and flucytosine, respectively. Eight of the nine *C. tropicalis* isolates had similar phenotypic and PCR fingerprinting (isolates were from two patient and the environment). In our study isolation of *Candida* species from environmental and the healthcare workers samples were rare.
Conclusion: The finding of this prospective study suggests that the nosocomial acquisition of *Candida* species as we found with *C. tropicalis* is not uncommon and may be due to the exogenous acquisition. Further prospective studies are needed to define more clearly the reservoirs of infection, the mode of transmission and the optimal measures for preventing spread of *Candida* species.

P822 Changing pattern of *Candida* spp. isolated from neutropenic patients in a single institution

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Background: Changing patterns of *Candida* species causing fungal infections in neutropenic patients along with decreased azole susceptibility is now reported with increasing frequency. We undertook this study to examine the distribution of clinical isolates of *Candida* species from infections of neutropenic patients.
Methods: The study was carried out in the periods of 1999–2001 and 2002–2003 *Candida* isolates from all samples of febrile neutropenic patients were identified by conventional methods, germ tube testing being the first step followed by morphology on cornmeal Tween 80 agar and carbohydrates assimilation and fermentation results by using ID32C (BioMerieux, France).
Results: A total 174 of *Candida* spp. were isolated from 115 patients. 127 of them recovered in the 1999–2001 period and 47 of them recovered in the 2002–2003 period. The distrubution of *Candida* species fotr the first study and second period was as following; *C. albicans* for 68.8 and 48.9%, *C. glabrata* 14.3 and 4.2%, *C. tropicalis* 10.4 and 8.5%, *C. krusei* 3.9 and 2.1%, *C. kefyr* 3.9 and 27.6%.
Conclusions: Increase in the prevalance of nonalbicans *Candida* spp. was obseved in the latter study period. Continuous surveys of the distrubution of *Candida* spp. in an institution are warranted.

P823 Colonization of intensive care and hemato-oncologic patients by *Candida* species: is there a trend towards more frequent isolation of non-albicans species?

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Objectives: According to published surveillance data, the etiology of Candida bloodstream infections has shown a significant worldwide shift towards nonalbicans species, at least during the late eighties and early nineties. However, most studies were not able to detect a further increase during the late nineties. The aim of the present study was to monitor the relative prevalence of different *Candida* species not recovered from blood cultures, but from surveillance cultures of intensive care patients and hemato-oncologic patients during a 5-year period (1997–2001) in a tertiary-care hospital in South-West Germany (1500 beds).
Methods: Clinical and surveillance specimens (upper respiratory tract swabs, lower respiratory tract specimens, gastric juice, anal swabs, urinary tract samples and genitourinary tract swabs, etc.) were cultured on Candida-II-agar (heipha, Heidelberg, Germany). Isolates were typed by Chromagar Candida (Mast Diagnostica, Reinfeld, Germany) and/or ATB ID 32 C (BioMérieux, Marcy-L'Etoile, France).
Results: From 1997 to 2001, after elimination of multicopy strains, a total of 8000 Candida isolates were obtained. The following significant trends ($P < 0.01$) were observed: *C. glabrata* increased from 14.9% of all urinary tract isolates (1997) to 46.8% (2001). *C. tropicalis* increased from 3.0% of all upper respiratory tract isolates (1997) to 7.3% (2001). There were no differences between intensive care patients and hemato-oncologic patients. In 2001, relative prevalences of the most prevalent species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*) were: upper respiratory tract 74.5, 13.9, 7.3, and 2.2%; lower respiratory tract 59.5, 32.0, 4.7, and 2.0%; gastrointestinal specimens 63.2, 29.1, 3.2, and 3.2%; urinary specimens 45.2, 46.8, 4.0, and 1.6%.
Conclusion: During the study period, the following significant trends were noted: For urinary tract specimens, a major shift to *C. glabrata* was detected, now being the most prevalent species in these specimens. For upper respiratory tract specimens, a minor increase in the relative prevalence of *C. tropicalis* was noted.

P824 *Candida albicans* versus *Candida nonalbicans* candidemia among the critically ill patients in a tertiary hospital, Greece

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Objectives: This observentional prospective study was designed to compare the frequencies of *Candida albicans* and *Candida nonalbicans* candidemia cases in Intensive Care Unit (ICU) non-immunocompromised patients, depict the associated risk factors and appraise respective mortality rates.
Methods: Observations on 327 patients in the ICU (January 2001–December 2002), comprised data on age, sex, length of ICU stay (LOS), duration of Mechanical Ventilation (MV), Acute Physiology and Chronic Health Evaluation Score(APACHE II), on admission. Predisposing factors for candidemia, such as diabetes, duration of antibacterial chemotherapy regimens, candiduria, steroid treatment, total parenteral nutrition (TPN), presence of central venous

catheter (CVC), duration of MV(>7 days), prior major surgery and administration of antifungal prophylaxis were also recorded. Candidemia was defined by at least two positive blood cultures. The point in time of candidemia, Candida septic shock and outcome were also assessed. Statistical analysis encompassed *t*-test for quantitative parameters and chi-square test for categorical data. Probability (P) < 0.05 was considered statistical significant.

Results: Candidemia was diagnosed in 55/327 patients (16.8%). *C. albicans* was responsible for 35 (63.6%) cases (Group A) while *C. nonalbicans* species for 20 (36.4%) cases (Group B). In both groups no differences were detected on age, sex, LOS, duration of MV and APACHE II score($P > 0.05$). Candidemia due to *C. nonalbicans* species was significantly more frequent, in patients with positive CVC cultures ($P < 0.003$), in those with candiduria episodes ($P < 0.0001$)and in patients undergoing steroid therapy ($P < 0.014$). In these three groups of patients the commonest bloodstream isolates were *C. glabrata* 8 (40%), *C. parapsilosis* 5 (25%) followed by *C. tropicalis* 4 (20%, *C. krusei* 2 (10%) and *C. guillermondii* 1 (5%). Crude mortality was higher in Group B ($P < 0.009$). No difference was detected in the mortality attributed to candidemia (Group A 10.4% vs. Group B 10%, $P > 0.5$).

Conclusions: CVC, candiduria and steroid treatment were the commonest predisposing factors for *C. nonalbicans* candidemia. Though the crude mortality was higher among patients with *C. nonalbicans* candidemia, the overall candidemia mortality was not significantly different between the two groups of patients.

P825 Frequency, risk factors and outcome of candidemia in critically ill patients

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Brussels, B

Objectives: *Candida* spp. are the most common cause of fungal infections in critically ill patients. We conducted a prospective study to compare the morbidity and mortality of patients infected with *Candida* spp. with patients infected by other pathogens.

Methods: The study included 949 patients who stayed more than 24 h in a mixed 31 bed Intensive Care Department (ICU) of a tertiary care hospital; 31 patients with diagnosed candidemia were compared with 249 patients with bacterial infections. The analyzed variables included previous hospital stay, length of ICU stay (LOS), need and duration of mechanical ventilation (MV) and hemodialysis or hemofiltration, APACHE (Acute Physiologic and Chronic Health Evaluation) II score, SOFA (Sequential Organ Failure Assessment) score at admission, SOFA maximum score during patients ICU stay, SOFA score on the day of infection diagnosis, antibiotic use, type of nutritional support, association with another infection, immune response, C-reactive protein, procalcitonin levels, number of medical devices, and mortality.

Results: Of 280 infected patients, 31(11%) were infected with *Candida* spp., including 18 (58%) *Candida albicans*, and 13 (42%) *Candida nonalbicans* spp. (*C. glabrata* in 5 cases, *C. parapsilosis* in 3 cases, *C. krusei*, *C. tropicalis*, *C. guilliermondii*, *C. lusitaniae* in 1 case, respectively). Candidemia was always associated with other bacterial strains and among these with methicillin-resistant *Staphylococcus aureus* (MRSA) in 11 (35%) patients. Both the degree of morbidity assessed by APACHE II and SOFA scores and mortality were higher in patients with candidemia than in other infected patients. Univariate logistic regression revealed the degree of morbidity, LOS, antibiotic use, duration of MV, and the number of medical devices as independent factors associated with Candida infections ($P < 0.05$). Multivariate logistic regression revealed only the LOS as independent predictor for Candida infection ($P = 0.03$). There was a trend toward a higher mortality rate in patients with candidemia (48% vs. 37% in bacterial infections), but it was not statistically significant ($P = 0.09$). Infection with Candida per se was not an independent factor to predict mortality.

Conclusions: Bloodstream infections with *Candida* spp. are common in ICU patients, they are associated with other bacterial infections and show high mortality rates. A longer ICU stay is the best predictive factor for candidemia.

P826 The outbreak of nosocomial fungemia caused by *Candida pelliculosa*

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Objective: *Candida pelliculosa* is a rare cause of fungemia. Between April 1999 and December 2001, 167 cases of *C. pelliculosa* fungemia occurred in the Newborn Unit. We investigated the antifungal susceptibility for these species, and using orally nystatin for prophylaxis.

Methods: Between April 1999 and December 2001, BACTEC 9240 detected 184 bloods, three cerebrospinal fluids, and one peritoneal fluid culture obtained from the Newborn Unit as positive. Twenty-one of 188 cultures were isolated same patients. A Gram-strained smear of the positive bottles revealed yeasts, which were subsequently identified as *C. pelliculosa*. Antifungal susceptibility to amphotericin B, fluconazole, ketoconazole, itraconazole, and nystatin was determinate by using National Committee for Clinical Laboratory Standards microdilution reference methods.

Results: Results of environmental cultures study including hands of personnel, and all areas of the Newborn Unit to which patients had potentially been exposed were negative. After July 2000, orally nystatin was started each infant for prophylaxis in the Newborn Unit. During follow-up (July 2000) only six candidemia occurred that were caused by *C. pelliculosa*. After December 2001, this species was not isolated in this Unit. The minimum inhibitory concentrations (MICs) for *C. pelliculosa* species were found in 0.06–0.125 mg/mL for amphotericin B; 1–4 mg/mL for fluconazole; 0.03–0.25 mg/mL for ketoconazole; 0.03–0.25 for itraconazole; 0.5–2 for nystatin. Of the 167 cases, 51 were retrospective analyzed according to antifungal treatment. Systemic antifungal agents had been used after the occurrence of candidemia in 42 cases: amphotericin B, fluconazole (22), and amphotericin B subsequently fluconazole (nine). Only nine patients not received antifungal treatment. Patients who received treatment had higher improved than did not treated patients (62% vs. 33%). The overall crude mortality rate was 43%.

Conclusion: We conclude that *C. pelliculosa* should be considered in the differential diagnosis of blood cultures and used orally nystatin for prophylaxis in the Newborn Unit.

P827 Laboratory investigation of fungal peritonitis in patients on continuous ambulatory peritoneal dialysis

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Objective: Fungal peritonitis is a relatively uncommon complication in patients on continuous ambulatory peritoneal dialysis (CAPD), but accounts for significant morbidity and mortality. We reviewed the etiology and antifungal susceptibility of the fungi isolated from peritoneal dialysis effluent of patients on CAPD at our hospital in the last 11 years (1992–2002).

Methods: Fungal isolates were identified using standard mycologic laboratory methods and tested prospectively for susceptibility to fluconazole (FCZ), flucytosine (5FC) and amphotericin B (AMB). MIC tests have evolved over the 11 years reflecting best practice at the time of use.

Results: There were 43 episodes of fungal peritonitis in 41 patients during this period. The fungi isolated were *Candida albicans* (18), *Candida parapsilosis* (17), *Candida tropicalis* (2), *Candida guilliermondii* (2), *Candida kefyr* (1), *Candida glabrata* (1), *Candida pelliculosa* (1) and *Saccharomyces cerevisiae* (1).

Conclusions: *C. albicans* and *C. parapsilosis* were the predominant fungi isolated constituting 81.4% of fungal peritonitis in our hospital. In general, both species were susceptible to FCZ, 5FC and AMB. There was a high degree of FCZ resistance (62.5%) among the other fungi. Current guidelines (Year 2000) from the International Society for Peritoneal Dialysis recommend initial imidazole/triazole and flucytosine without catheter removal when fungi are identified by Gram stain or culture and use fungal sensitivities to guide treatment for 4–6 weeks. In the absence of clinical improvement, catheter removal is warranted after 4–7 days of therapy. Therefore, identification and antifungal susceptibility testing of fungi isolated from peritoneal effluent is essential for optimal management of fungal peritonitis in patients on CAPD.

Bacterial infections in children

P828 *Chlamydophila pneumoniae* infections in infants

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Objectives: The aim of the study was to investigate the prevalence of *Chlamydophila pneumoniae* infections in infants hospitalized due to respiratory tract diseases.
Methods: Serum samples from 158 hospitalized infants from 1 to 36 months old-were examinated. Eighty-three children presented with clinical symptoms of various respiratory tract infections, 75 suffered from other diseases. Levels of specific *Chlamydophila pneumoniae* serum IgM, IgG and IgA antibodies were determined with ELISA method (Elegance *Chlamydia pneumoniae* IgG/IgA ELISA, Bioclone, Australia; *Chlamydia pneumoniae* IgM ELISA Vircell, Spain). Antibodies to *Mycoplasma pneumoniae* were measured using ELISA test (MRL Diagnostic, USA).
Results: Among 83 infants with respiratory tract infections, *C. pneumoniae* specific IgM antibodies were detected in 12 (14.5%)(in 9 infants with bronchitis and in 3 ones with upper airways infection). Specific IgG antibodies were found in 2 infants (9 and 12 months old) as well as in 6 ones under age of 6 months. No specific IgA antibodies were detected. Among 75 children with other diseases, 6 (8.0%) had *C. pneumoniae* specific IgM antibodies and 1 (19 months old) had *C. pneumoniae* specific IgG antibodies. Mixed *C. pneumoniae* and *M. pneumoniae* infections were found in 8 (9.6%) of children. Six of them suffered from bronchitis. Infants were infected with *C. pneumoniae* most often in October, November and December.
Conclusions:
1. *C. pneumoniae* infections are seen in infants younger then three years.
2. *C. pneumoniae* infections are seasonal, mostly occur in autumn.
3. Mixed *C. pneumoniae* and *M. pneumoniae* infections are present especially in infants with bronchitis

P829 Urinary tract infection in boys under the age of 5

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Objective: To determine the frequency of the urinary tract infection(UTI) and its etiology.
Methods: 79 boys under the age of 5 years with a suspected urinary tract infection were addressed by the pediatrists to our laboratory, during our study period of one year (May 1997–May 1998).The strains isolated were identified with API 20E gallery for the Gram-negative bacilli and the classical gallery for the Gram-positive cocci. The antibiotic susceptibility was determined by the agar diffusion method according to NCCLS. The antibiotics effective on Gram-negative bacilli tested were: Ampicillin, Amoxicillin/clavulanic acid, Ticarcillin, cefalotin, Cefoxitin, Cefotaxim, Gentamycin, Amikacin, Nalidixic acid and sulfamethoxazol.
Results: Ten boys out of 79 (12.62%) presented a bacteriuria with pyuria and 9 boys out of 79 (11.39%) a bacteriuria without a pyuria.100 urine samples from the 79 boys were tested of which 49 had < 1000 UFC/mL, 22 had $> 100\,000$ UFC/mL and 29 had 1000–10000 UFC/mL(contaminated). 40% of the boys who presented a UTI were under the age of a year. Three boys out of 4 presenting a malformative urinary tract were infected by the bacteria *Proteus mirabilis*. *P. mirabilis* was the most frequent agent isolated in the bacteriuria with pyuria (50%) and without pyuria (58.33%).The strains of *P. mirabilis* usually susceptible were highly resistant 100% to Ampicillin, 90% to Ticarcillin 81.8% to pipercillin and 54.5% to cotrimoxazol.
Conclusion: The urinary tract infection is frequent in boys (24%) in our study (19/79) and is often associated to a malformative urinary tract (4/10). *P. mirabilis* is the main agent isolated and its resistance to betalactams and cotrimoxazol increased more and more, so these antibiotic could not be anymore the initial antibiotic therapy. The susceptibility test is needed to treat correctly this serious infection.

P830 *E. coli* causing urinary tract infection in Greek children. Resistance to antibiotics and virulence factors

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Background: Urinary tract infections (UTI) affect a large number of the world population. Uropathogenic *E. coli* (UPEC) is the cause of most of these infections. Virulence factors associated with UPEC include several toxins (hemolysin, cytotoxin necrotizing factor), capsule, a number of adhesive organelles, etc.
Objectives: To determine the antibiotic resistance of *E. coli* producing UTI in children, as well as to detect and determine the frequency of virulence factors produced by this microorganism.
Methods: One hundred fifty two *E. coli* isolates from urine cultures of children with UTI, aged between 0 and 14 years, were studied in our laboratory, during one year's period (1/4/2001–31/3/2002). Resistance to antibiotics was examined according to NCCLS instructions. All strains were further examined for the production of hemolysin, K1 antigen (Directigen, Beckton – Dickinson) and expression of pili by agglutination of human O red blood cells.
Results: Resistance to antibiotics was as follows: ampicillin 50%, cephalothin 32.2% amoxycillin + clavulanic acid 5.3%, cefoxitin 1.3%, cefuroxime 7.9%, cefotaxime 5.3%, gentamicin 7.9%, netilmicin 4.6%, trimethoprim-sulfamethoxazole 27%, nitrofurantoin 0.6%, ciprofloxacin 1.3%, norfloxacin 1.3%. There were no strains resistant to imipenem. Conserning virulence factors, 28.2% of strains produced hemolysin, only 16.1% carried K1 antigen and 53.2% produced HRBC agglutinating pili. In all cases, agglutination was mannose-resistant, indicating the presence of P or Dr pili.
Conclusion: *E. coli* causing UTIs in children from Greece is often resistant to ampicillin, cephalothin and trimethoprim-sulfamethoxazole. Resistance to second and third generation cephalosporins, aminoglycosides and quinolones remains relatively low. Careful use of antibiotics is needed to avoid further increase of resistance. Pili is the most frequent virulence factor detected. Detection of virulence factor genes in these strains by molecular techniques is considered necessary. The above is being performed and will be announced at a later date.

P831 Paediatric infective endocarditis: a review of 22 cases

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Objectives: To evaluate the epidemiology, risk factors, clinical features, echocardiographic and laboratory characteristics of infective endocarditis in pediatrics age group.
Methods: A total of 22 pediatric patients diagnosed with infective endocarditis between January 1996 and November 2001 at The Aga Khan University, Karachi, Pakistan were included in this review.
Results: Of the 22 patients with infective endocarditis, male to female ratio was $\sim 1:1$ (Male 45.5%, Female 54.5%).
Patients: Age ranged from 1 month to 14 years with a mean age of 5.8 ± 4.8 years. Twenty patients (91%) presented with fever and an equal number had heart murmurs at the time of evaluation. Splenomegaly was found in only 4 patients (18%). A distant focus of infection was present in 5 (23%) patients and pneumonia was diagnosed in two of them (9%). Underlying congenital heart disease was present in 19 (86%) patients while only one had rheumatic heart disease. Ten patients (45.5%) had native valve endocarditis while a VSD was involved in 5 (23%) patients. A history of cardiac surgery was present in 3(14%). On echocardiography, vegetations were seen in 12 patients (55%). The commonest sites involved were mitral valve (23%) and aortic valve (14%). Ten (45.5%) patients did not show any evidence of vegetations on echocardiography. Blood cultures were positive in 11 (50%) patients with Streptococci (27%) and Staphylococci (9%) being the commonest organisms isolated. Viridans group streptococci were found to be the commonest species (18%)

responsible for infective endocarditis. Eleven (50%) patients did not grow any organism on their blood cultures. Neurological complications were present in 6 (27%) patients and the commonest of them were cerebral embolism (9%) and seizures (9%). Other complications included hemolysis (13%), hematuria (9%), and renal failure (9%). Surgical interventions were required in 2 (9%) patients. Inpatients mortality was 27.3%. Thirteen (59%) patients were alive without the disease (mean follow-up of 53 ± 48 weeks). Lost to follow up rate was found to be 4.5%.
Conclusions: This study demonstrates that fever and a heart murmur are common presenting features of infective endocarditis in pediatric population. Almost all of the patients with infective endocarditis have congenital heart disease (VSD being the commonest). Neurological complications may be the presenting feature of infective endocarditis in as many as one-fourth of the cases.

P832 Relationship between Lewis antigen and other virulence factors in pediatric *Helicobacter pylori* isolates

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Aim: The aim of this study was to determine the occurrence and relationship between the presence of Lewis antigen and other virulence factors (cagA and vacA) in *H. pylori* strains obtained from pediatric patients.
Introduction: The inflammatory response is a primary host defence mechanism against an invading pathogen. It is known that CagA + VacA + strains of *Helicobacter pylori* (Hp) are associated with enhanced inflammatory responses and mucosal damage. In the other hand, Hp lipopolysaccharides (LPS), the O-specific chain, mimics Lewis blood group antigens in structure (present in the gastric mucosa). So the expression of Lewis antigens on the bacterial surface may camouflage the bacterium and aid survival of Hp.
Methods: Fifty *H. pylori* strains were obtained from pediatric patients attending to the Gastroenterology Unit due to different symptomatology. Upper endoscopy was performed and biopsy cultured following standard methodology. Strains were conserved at -70°C until used. Lipopolysaccharide (LPS) was extracted from a 48-h culture by mini-phenol water. Detection of Lewis antigen was determined by a standard serodot method using monoclonal anti-LewisX, -LewisY and peroxidase-labelled secondary antibodies. DNA was extracted from a 48-h culture and PCR performed to detect cagA gene, and vacA s1- and s2-alleles.
Results: The frequency of *H. pylori* strains expressing LewisX was 34.7% and LewisY was 55%. Of the strains tested, 28.6% expressed LewisX and LewisY simultaneously and 38.8% no Lewis antigen. In 32.6% of strains, the expected cagA fragment was amplified by PCR; 34.8% carried the s1-allele and 65.2% the s2-allele. A correlation was observed between cagA positivity and the vacA s1-allele ($P < 0.01$), and between the presence of LewisX and LewisY ($P < 0.01$). Also, the presence of the cagA gene was associated with the presence of LewisX ($P < 0.01$) and LewisY ($P < 0.001$).
Conclusions: Of the pediatric-originating strains tested, a lower percentage expressed both LewisX and LewisY than previously reported in isolates from adults. Also, a low prevalence of cagA gene and vacA s1-allele was observed in strains from pediatric patients. Furthermore, occurrence of cagA was associated with vacA s1-allele and to LewisX and LewisY in this population.

P833 Prevalence and resistance of bacterial enteropathogens in children in Crete

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Objectives: To investigate the epidemiology and resistance rates to antibacterial agents of the enteropathogens responsible for bacterial gastroenteritis in children in Crete, Greece. Population-Methods: All positive stool cultures from hospitalized and outpatient children during the period January 1993 to December 2001 were included.
Results: One or more pathogens were identified in 1059 stool specimens. *Salmonellae* spp. were the most commonly isolated (36.6%), followed by *Campylobacter* spp. (32%), enteropathogenic *E. coli* (*EPEC*) (23.7%), *Yersiniae* spp. (5.3%), *Shigellae* spp. (1.9%) and *Aeromonas* spp. (0.7%). The overall resistance rates to antibacterial agents are presented in Table 1. The resistance rates of the pathogens to the antibiotics of choice were in general high. A significant drop of resistant strains of *Salmonellae* spp. to ampicillin was noted from the first to the third 3-year period of the study (40.2% vs. 15.7%; $P < 0.0001$; relative risk 2.56). A similar resistance decline was noted for *Campylobacter* spp. to erythromycin, although not at a significant level (21.7% vs. 12.9%; $P = 0.1054$; relative risk = 1.67).
Conclusions: In the area of the study, *Salmonella* spp. and *Campylobacter* spp. are the leading causes of bacterial gastroenteritis in childhood. The drop of resistance rates of *Salmonella* spp. and, to a lesser degree, of *Campylobacter* spp. to the antibiotics of first choice might be attributed to the more rational use of these antibiotics through the study period, however, the overall resistance of the enteropathogens remains high.

Table 1 Resistance rates of enteropathogens to usual antibacterial agents (%)

	Salmonellae	*Campylobacter*	*Yersiniae*	*EPEC*	*Shigellae*	*Aeromonas*
Ampicillin	31.1	ND	100	29	60	100
Cefotaxime	0	ND	0	0.4	0	0
Gentamicin	1.5	2.3	0	2.4	0	0
Chloramphenicol	6.2	7.9	3.5	13	45	0
Ciprofloxacin	0	42.5	0	1.6	0	0
Cotrimoxazole	3.3	69.5	1.7	17	60	40
Erythromycin	ND	19.3	ND	ND	ND	ND

P834 First occurrence of sorbitol-fermenting Shiga toxin-producing *E. coli* O157 in Austria

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Objectives: Shiga toxin-producing *E. coli* (STEC) have emerged worldwide as a cause of severe human diseases such as bloody diarrhea and the hemolytic uremic syndrome (HUS). First recognized in Germany in 1988, SF STEC O157:H- has been isolated from patients with diarrhea or HUS in the Czech Republic, Hungary, Finland and recently in Australia and Scotland. We report the first isolation of an SF STEC O157:H- in Austria.
Methods and results: A 34-month-old girl was admitted with HUS to a hospital in Salzburg on November 2, four days after onset of bloody diarrhea. On admission, laboratory investigation indicated anuric renal failure (plasma creatinine 7.5 mg/dL; normal range for this age 0.3–0.6 mg/dL) and hemolytic anemia (hemoglobin 10.9 g/dL; normal range for this age 11.5–14.0 mg/dL) with 5% fragmentocytes. The platelet count was normal (196,000/mm^3; normal range for this age 140,000–440,000/mm^3). Lactic dehydrogenase was highly elevated (1900 U/L; normal range 120–340 U/L). The child remained anuric despite furosemide therapy and veno-venous hemodiafiltration was initiated. A diagnostic kidney biopsy was performed on day 7 of hospitalization just before receiving positive culture results from the national reference laboratory. It is questionable whether diagnostic kidney biopsy should have been performed under these circumstances. After initiation of anesthesia, just before puncture an epileptic attack occurred, after biopsy uncontrollable bleeding necessitated unilateral nephrectomy on day 9. Pancreatic insufficiency with insulin dependent diabetes mellitus and indication for hemofiltration were still present on day 27, when the patient, who is presently on a waiting list for kidney-transplantation, was transferred to a specialized clinic abroad. Stool specimens gained on days 3 and 4 were tested at the local microbiology laboratory using SMAC plates and reported as 'no STEC found', but yielded tellurite sensitive and beta-glucuronidase positive SF STEC O157:H-, indistinguishable from the so-called Bavarian outbreak clone by PFGE and ribotyping, when tested at the national reference laboratory using toxin ELISA and PCR based screening for stx genes.
Conclusions: Laboratories should be cautious of overly relying on visual identification of *E. coli* O157 on both sorbitol MacConkey agar (SMAC) and cefixime-tellurite (CT)-SMAC plates in patients presenting with clinical symptoms suggestive of an STEC infection.

P835 Secondary lactase deficiency in Japanease infants due to respiratory infections

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The adult of Japanese can not secrete lactase, which is the primary lactase deficiency, while, the infant of Japanese can secrete lactase into the intestine.

Diarrhea, however, is often observed even in infant who is suffered from infection except for digestive tract. Secondary lactase deficiency is considered as this disease state. In this study, the frequency of secondary lactase deficiency was examined as a cause of the diarrhea with respiratory infection in infancy.
Materials and methods: The 82 patients, who were suffered from the respiratory infections complicated with watery diarrhea, were included in this study. All patients were necessary to hospitalize for the intravenous fluid therapy. All feces were examined the reducing sugar, enteropathogenic bacteriae and antigens of Rota virus and Adeno virus.
Results and conclusions: The reducing sugar was detected in feces from 43 out of 82 patients, that is, these patients were secondary lactase deficiency. No feces showed positive reaction of enteropathogenic bacteriae or of antigens of Rota virus or Adeno virus. There was no relationship between the positive rate of the reducing sugar in feces and the degree or the type of respiratory infections. The reducing sugar in feces was more frequently detected in the patients belonged in younger age group. It may be concluded that there is an individual difference on capacity of lactase secretion even in the infant of Japanese.

P836 Asessment of Cefepime monotherapy vs. combined therapy with Ceftriaxone and Aminoglicosyde in oncologic children and adolescents with febrile neutropenia

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Objective: Assessment of cefepime monotherapy's efficacy in the treatment of febrile neutropenia in children and adolescents, comparing with combined therapy with ceftriaxone and aminoglicosyde (amikacin or gentamicin).
Methods: Retropective assessment of oncologic patients admitted with neutropenia (granulocytes <500) and fever (axillar temperature higuer than 38.5°C).We defined as therapeutic success patients that become without fever after 5 days. Therapeutic failure was defined as clinical deterioration or grown of resistant bacteria during treatment.
Results: We have analyzed 51 patients who were admitted for 67 episodes of neutropenia and fever. Thirty-seven episodes (50%) had negative urine and blood culture and 37 (50%) had a positive urine and/or blood culture, with a total of 49 positive cultures. The most frequent isolates from blood cultures were *Klebsiella pneumoniae* (29.4%), *Acinetobacter* sp. (17.6%) and *Enterobacter* sp. (11.7%); and from urine cultures were *Pseudomonas aeruginosa* (30%) and *Proteus* sp. (16.6%). We analyzed the antibiotic use in 67 episodes of febrile neutropenia. Ceftriaxone and aminoglicoside were used in 29 episodes (43.2%) and cefepime was used in the remaining 38 episodes (56.7%). The groups were comparable in relation to age, duration of fever and neutrophil count. In the combined therapy group there were 18 therapeutic failures (62%) and in the monotherapy group, 12 failures (31.5%) ($P = 0{,}01$).
Conclusion: In our series, cefepime monotherapy was superior to the combination therapy with ceftriaxone and aminoglicoside in the treatment of febrile neutropenia. Most of our isolates were Gram-negative agents (77.5%). We observed a low rate of isolation of Gram-positive agents (12.2%) and fungi (10.2%), even though febrile neutropenia is a important risk factor for infections with these agents.

P837 Eosinophilic cationic protein in serum of children with acute respiratory diseases

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Objective: to define the correlation between serum Eosinophilic cationic protein (ECP) levels and the inflammatory procress of the respiratory tract during infections or asthmatic reactions.
Methods: We studied 56 children (24 girls and 32 boys), aged 3 months–13 years, with respiratory tract infections diagnosed by radiography and 15 with asthmatic reactions. Serum ECP levels were mesured at acute phase of disease by using FEIA method. IgM or fourfold IgG antibodies titers against respiratory viruses or *Mycoplasma pneumoniae* and antigen detection by using EIA and ELFA.
Results: 24 children (42.8%) presented concetrations of ECP > 15 g/mL and 32 (57.1%) ECP < 15 g/mL. Of the children with high levels, 37.5% had respiratory tract infection with asthmatic reaction, 29.15% asthma attack, 12.5% respiratory tract infection, 8.3% laryngitis and 12.5% broncholitis with RSV(antigen positive). Of the children with ECP low levels 31.25% had respiratory tract infection, 28.12% infection with asthmatic reaction, 28.12% asthma attack, 6.25% laryngitis and 6.25% broncholitis (Antigen RSV negative).
Conclusions: (1) High serum ECP concetrations are found in a greater percentage of children with asthmatic reaction with or without infection compated to chidren with respiratory tract infection ($P = 0001$). (2) The results suggest that the inflammation in RSV bronchiolitis differs from that induced by other viruses or *Mycoplasma pneumoniae*.

P838 Chronic mediastinitis in children after cardiac surgery: a complication observed in developing countries?

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Our hospital has a cardio-surgical activity of 300 children operated on a year, 60% of which are Algerian. We report two cases of chronic mediastinitis complicating cardiac surgery in Algerian patients. The first case is a 9-year-old girl admitted for the cure of a retrosternal fistula that had persisted during five years after cardiac surgery performed in her country. On admission, *Staphylococcus aureus* was grown from the fistula. The child underwent several debridements and surgical drainage of the mediastinum. She presented a mediastinal super infection with *Pseudomonas aeruginosa* at the time of an epidemic in the intensive care unit. Additionally to the surgical treatment, she received a prolonged course of antibiotics. The second case is a girl who underwent a former cardiac surgery at the age of two, complicated by a sternitis that lasted for two years. At the age of seven, she was admitted for the last stage of her cardiac correction. On the third postoperative day, she developed sepsis and *P. aeruginosa* was grown from the sternal wound and blood cultures. She was treated by surgical debridement and drainage and antimicrobial intravenous therapy. Ten days later fever increased again and a chest CT showed a retrosternal collection. She had a new surgery that evidenced an aortic pseudoaneurysm. The ascending aorta was partially replaced by a homograft aortic root. Cultures from the aorta remained negative. She was treated with a 3-month intravenous antimicrobial regimen followed by a prolonged oral ciprofloxacin treatment. These two patients had a favourable outcome after aggressive surgical treatment combined with prolonged antimicrobial therapy. Mediastinitis following cardiac surgery are uncommon in children operated on in developed countries. Optimal antimicrobial regimen and duration of treatment are not well established.

Therapeutic issues in HIV infection

P839 Modifications induced by antiretroviral therapy on cytokine phenotype 1 and 2 during HIV infection

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Objectives: Our aim was to verify possible alterations in vitro secretion of IFN-gamma and IL-4 by mononuclear cells in the peripheral blood (PBMCs) of HIV patients in different stage of the disease, both naive and undergoing highly active antiretroviral therapy (HAART) to compare the results obtained in two groups and in 12 patients after one year of antiretroviral therapy.
Methods: Fifty two HIV-positive patients (39 males and 13 females with a mean age of 40.5 ± 1.4 years) were enrolled in this study. All patients were classified on the basis of treatment (therapy vs. no therapy), on the basis of the clinical stage (A, B or C) as defined by the Center for Disease Control (CDC), and on the basis of the viro-immunological condition (lymphocyte CD4 counts and blood HIV-RNA). Briefly, Th1 and Th2 cytokines (IFN-gamma and IL-4, respectively) were detected in surnatant samples by ELISA kit (R&D Systems, Minneapolis, MN) derivated by cell cultures incubated for 48 h and samples were stimulated by anti-CD3, Phytohemoagglutin-P (PHA), *E. coli* B04/035 Lipopolysaccharide (LPS) in all selected subjects. In 12 patients a second analysis was performed after 12 months.
Results: A progressive decrease in the baseline production of IFN-gamma was observed from patients with CD4 > 500 to patients with CD4 < 200. In contrast, the IL-4 production was increased in the PHA-stimulated cultures both in clinical and immunological progression. IL-4 secretion was lower in the treated subjects compared with the naive ones in PHA-stimulated cultures. Treatment caused an increase of the IFN-gamma/IL-4 ratio and a 12-months' follow-up study in 12 patients showed a significant decrease of IL-4 and an increase of IFN-gamma.
Conclusion: Our data confirm the alteration of Th1/Th2 balance reported by many authors and support the hypothesis of a cytokine switch in the progression of HIV-1 disease and the important role of the antiretroviral therapy in the restoring of the balance of type 1 and 2 patterns.

P840 Indinavir vs. Indinavir-boosted by Ritonavir plus 2 new NRTIs in rescue of dual NRTI regimen virological failures

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Background: Broad use of dual NRTI therapy has resulted in increasing numbers of patients failing this single antiretroviral class approach. Protease inhibitor (PI) boosted regimens, including PI-combinations such as IDV/RTV, offer rescue options for these patients.
Methods: This prospective, open-label, 24 week. study conducted in Brazil randomized 78 patients having failed a dual NRTI regimen to group A: IDV 800 mg tid ($n = 38$) or group B: IDV 800 mg/RTV 100 mg bid ($n = 40$). Both previous NRTIs were replaced. Local brands of NRTIs were used as switch agents due to reimbursement requirements. Efficacy was determined by viral load (VL) reduction and CD4 increase. Safety, tolerability, and compliance were also assessed.
Results: The most frequent NRTI prior to switch was ZDV (A = 95%; B = 80%) followed by DDI (A = 61%; B = 48%). The most frequent replacement was another thymidine analogue, D4T (A = 100%); B = 94%) and the second most frequent was 3TC (A = 87%; B = 77%). Baseline median VLs (cps/mL) were: A = 18K; B = 15K and median CD4 (cells/mm^3) were: A = 374; B = 411. Observed data at week 24, 42.4% of both A and B achieved VLs < 400, median log decreases of A = −0.71 and B = −1.13 were recorded, and CD4 increases of A = +69 and B = +61. Using the criteria of VL < 400 or > 1.5 log decrease, 60.5% of A and 52.5% of B could be classified as responders. Of those with baseline VL > 50K, 8% of A and 23% of B achieved VL < 400. Discontinuations were slightly higher in B (18%) compared with A (11%) as were discontinuations due to AEs in B (15%) vs. A (3%). The incidence of nephrolithiasis was the same in both arms (11/100 pt./year.). Mean PI drug compliance percentages were A-IDV = 95.9 ± 6.0; B-IDV = 94.1 ± 15.0 and RTV = 91.0 ± 20.0 at week 12 and A-IDV = 96.0 ± 6.2; B-IDV = 95.4 ± 12.5 and RTV = 92.7 ± 17.1 at week 24.
Conclusions: Similar overall rescue results were achieved with IDV tid and IDV/RTV bid + 2 new NRTIs for patients having virologically failed dual NRTI regimens. Rescue was lower for patients with higher VLs at baseline. In these cases, the boosted IDV regimen tended to afford greater success. Two possible confounding issues regarding the IDV/RTV regimens were lower RTV compliance and higher discontinuations due to AEs. Further assessments are in progress to determine whether the PI may have been the major contributor to regimen potency and NRTI-resistance may have compromised rescue.

P841 Characterization of viro-immunological responses in a closely followed cohort of heavily pretreated patients: evidence from the GenPheRex Study (MaSTeR Cohort)

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Background: Factors associated to viro-immunological discordant trends have been poorly investigated in cohorts of heavily pretreated patients (pts), mostly using categorical heterogeneous viro-immunological end-points.
Methods: One hundred fifty nine patients in a prospective study of real- vs. virtual-phenotype who experienced ≥2 years antiretroviral therapy with ≥6 drugs have been studied. First, univariate and multivariate logistic regression was used to assess risk factors for categorical discordant responses ceasing follow-up at week 32 since more pts were on the original drug combination after reasonable time to evaluate immune response. Second, complementary linear regression analysis was performed over the entire 48 weeks follow-up considering CD4+ and viral load (pVL) as continuous measures. ITT-LOCF was the primary analysis; OT analysis provided confirmation.
Results: Among 101 virological non responders (<1 Log10 c/mL pVL decrease), immunological discordance (≥100 CD4 + increase/mm^3) was observed in 63.5% of pts. In this group, baseline CD4+ > 200/mm^3 (OR 4.71, 95%CI 1.86–11.93; P: 0.001) and > 2 drug classes in the salvage therapy (OR 6.25, 95%CI 1.04–37.36; P: 0.045) were independently associated to the probability of discordant responses at logistic regression analysis. Among 58 virological responders, immunological discordances (< 100 CD4 + increase/mm^3) were observed in 58.6%. In this group, the following covariates were independently associated to discordant responses: baseline CD4+ > 200/mm^3 (OR 0.09, 95%CI 0.02–0.45; P: 0.003), randomization in the real-phenotype arm (OR 10.09, 95%CI 2.28–44.72; P: 0.002), and ≥3 experienced protease inhibitors (PIs) (OR 0.16, 95%CI 0.03–0.8; P: 0.026). Multivariable linear regression over the entire 48 week follow-up demonstrated significant correlation between absolute decrease in pVL and increase in CD4 + count (HR −28.06, 95%CI 35.32–20.79; P <0.001), but also PIs in the salvage regimen (HR 36.57, 95%CI 15.45–57.68; P: 0.001), > 8 months on treatment (HR 41.64, 95%CI 19.27–64.01; P <0.001) and randomization into the virtual-phenotype arm (HR 23.70, 95%CI 43.12–4.28; P: 0.017) correlated with better immune recovery.
Conclusions: In conclusion, these data suggest that therapy should be continued in heavily pretreated pts possibly including PIs. Hard-to-reach undetectability of the pVL is not essential to obtain immune recovery however, this is strongly favoured by the degree of pVL reduction that should be achieved.

P842 Extensive HIV-1 genotypic resistance pattern and evolution analyzes in patients failing to Nelfinavir-containing regimens

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Background: It has been suggested that nelfinavir (NFV) may not preclude efficacy of other PIs in the treatment sequencing however, this was mainly based on the sole consideration of signature mutations (D30N and L90M) in

the HIV protease gene. Moreover, it has been suggested that L90M and D30N are mutually exclusive on NFV containing regimens.
Methods: Extensive analysis of primary and secondary mutations or polymorphic substitutions in the HIV protease gene has been performed in patients (pts) who had first resistance testing while on NFV.
Results: Among 41 pts, 18 (43.9%) were PI naïve; D30N was detected from 10/41 (24.4%), L90M from 8/41 (19.5%), and D30N + L90M from one (2.4%); in 22/41 (53.7%) pts neither D30N nor L90M (D30N-/L90M-) were detected. D30N was found at higher frequency in PI naïve pts (70%), in contrast to L90M (12.5%). The unique patient with D30N + L90M had previous long-term experience with failing PIs (NFV, indinavir, saquinavir). The most frequent substitutions associated to D30N were S37N (70%), L63P (60%), E35D (50%), M36I (50%), I13V (40%), N88D (40%), N37D (30%), I62V (30%) and I93L (30%). All pts whose HIV carried D30N had $\geq$1 mutation common with other PIs (2/10 primary) among those listed in the last IAS algorithm, as well as pts with D30N-/L90M- resistance pattern (2/22 primary). Among pts whose HIV did not carry any primary mutations, mean number of secondary mutations was 1.6 (range 1–3) for pts with D30N and 1.8 (range 1–3) for pts with D30N-/L90M- pattern. In six patients who stayed on NFV despite virological failure, sequential resistance tests showed minimal variations in secondary mutations or polimorphisms up to 16 months, except from one patient with initial D30N-/L90M- pattern in whom L90M eventually emerged. Pts without L90M had complete initial virological response to alternative regimens including PIs.
Conclusions: Complete class cross-resistance through L90M in pts failing NFV was found at 1 : 6 ratio, mainly in those who had experience for other PIs however, secondary cross-resistant mutations were invariably present. The association between L90M and D30N was exceptionally found in one patient with experience for multiple PIs. Clinical cross-resistance to PIs in patients with D30N resistance pattern who were switched on to alternative PIs was not demonstrated. More studies are necessary to assess the clinical impact of secondary mutations on PI sequencing.

P843 Cessation of antiretroviral therapy in patients who started with previous guidelines: 48 weeks of follow-up

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Objectives: IAS-USA Updated Recommendations on Antiretroviral Therapy (Tarv), published July 2002, write that 'For persons who have already initiated therapy at higher CD4 cell count thresholds (e.g. 400, 450, or 500 cells/mL) and have had durable HIV RNA suppression and no adverse effects over periods of months to year, it is not clear whether it is safe to discontinue therapy.' (JAMA. 2002; 288 : 222–235). Our objective is to evaluate clinical, immunologic and virological course after cessation of Tarv in Patients (Ps) who started according to previous Guidelines at earlier stage of infection.
Methods: Perspective observational cohort study. 20 Ps (around 20% of all Ps on Tarv in our Unit) stopped Tarv; they were examined every 2 months and should restart Tarv if recommended by actual Guidelines.
Results: We examine 16 Ps (10 men, 6 woman) after at least 48 weeks of cessation. Ps, at moment of cessation, were between stage A1 and B2 (CDC 93); 10 Ps were on Tarv with 2 NRTIs; 3 Ps with 2 NRTIs + 1 NNRTI and 3 Ps with 2 NRTIs + 1 IP; 9/16 Ps had HIV-RNA < 200 c/mL. No opportunistic infections or malignancies occurred during follow up, there were 3 cases of Thrush and 1 case of Thrush + Oral hairy leukoplakia. No Ps restarted therapy according current DHSS Guidelines. In Table 1 we show immunologic and virological data (Table 1).

Table 1

Ps $n = 16$	START Tarv	STOP Tarv	24 weeks	48 weeks
CD4/mL mean (range)	508 (351–1.207)	664 (352–1.111)	568 (363–877)	523 (350–977)
HIV-RNA c/mL mean (range)	31.188 (<200–208.541)	1.039 (<200–14.800)	18.951 (450–49.000)	28.707 (684–51.500)

Conclusions: Our results suggest that in Ps who started therapy according to previous Guidelines (marginal indication to therapy) is possible to stop Tarv without relevant clinical, immunologic and virological risk; with improve of the quality of live in our Ps; elimination of toxic effects of drugs; elimination about risk of drug resistance (especially in Ps with sub optimal therapy and with incomplete viral suppression); decrease of cost.
Caution: Our study was performed in a little cohort and for relatively short follow up. We need a larger perspective trial.

P844 Effect of recombinant human growth hormone (r-hGH) on the activity of antiretroviral agents against wild-type and resistant human immunodeficiency virus-1 (HIV-1)

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Objective: The objective of our study was to assess the effect of r-hGH on the ability of approved nucleoside, nucleotide, non-nucleoside reverse transcriptase inhibitors and protease inhibitors to inhibit HIV-1 wild-type and resistant viruses in vitro.
Methods: These studies were performed by growing either wild-type or drug-resistant, mutated variants of HIV-1 in peripheral blood mononuclear cells (PBMC) in various concentrations of the nucleoside analogue reverse transcriptase (RT) inhibitor (NRTI) Abacavir, the nucleotide RT inhibitor Tenofovir, the non-nucleoside RT inhibitors (NNRTIs) Efavirenz, Delavirdine and Nevirapine, and the protease inhibitors (PIs) Nelfinavir, Amprenavir and Lopinavir in order to determine drug concentrations that inhibit viral replication by 50% (IC 50 values) for each compound. As a means of evaluating the potential effect of r-hGH (Serostim) on viral replication, this product was included in the tissue medium culture at concentrations of either 10 or 50 ng/mL.
Results: We have shown that r-hGH, at either 10 or 50 ng/mL over 48 h, exerted insignificant effects on viral replication levels (p24 antigen) after 7 days in regard to each one of the drug-resistant viruses that were studied. Furthermore, the responsiveness of wild-type variants against each of the antiviral drugs was not affected by the addition of r-hGH at either of the two tested concentrations (10 or 50 ng/mL). Also, the presence of r-hGH did not significantly alter the IC 50 values of the eight compounds that were studied using viral isolates that displayed resistance against any of NRTIs, NNRTIs, and/or PIs. This included viruses that were multiply drug resistant (MDR).
Conclusion: The anti-HIV effects of commonly used antiretroviral drugs in PMBC were not altered or inhibited by r-hGH. These tissue culture data provide no evidence that the use of r-hGH in HIV-infected people who are undergoing active antiretroviral therapy carry a risk of promoting viral replication.

P845 High-dose chemotherapy and autologous peripheral blood stem cell transplantation as salvage treatment for HIV-associated lymphoma

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Background: Availability of effective HAART has allowed for aggressive therapeutic approaches to be postulated for relapsing or not responder HIV-Ly however, no data currently exist on related clinical outcome.
Methods: Patients (pts) with relapsing or refractory (< 50% debulking after 1 month of standard chemotherapy) HIV-Ly were considered for inclusion in this salvage program consisting of ablative chemotherapy (MINE combination and/or high dose cyclophosphamide) followed by CD34+ stem cell apheresis after G-CSF stimulation, HDCT and subsequent CD34+ transplant. Patients with active mayor opportunistic infections or CSN lymphoma were excluded from this program.
Results: Up to September 2002, 10 pts entered the program; 7 Hodgkin Disease (HD, 3 at 1st relapse, 2 at 2nd relapse, and 2 refractory) and 3 NHD (all at 1st relapse). Median age was 40 years (range 31–56), median CD4+ count 196/cmm (101–451); disease stage as by W-F was II (n: 1), III (n: 3) and IV (n: 6); bone marrow involvement was found in 4 pts. After a median of 3 (2–3) apheresis, a median of 5.9 (4.1–8.3) × 106/kg CD34 + cells were collected; only 2 pts failed to mobilize enough CD34 + cells for transplant. Two pts with advanced HD died before transplant due to disease progression. After apheresis, the six remaining pts underwent HDCT with BEAM (BCNU 300 mg/sm, melphalan 140 mg/sm, vepesid 200 mg/sm and ARA-C 200 mg/sm) followed by CD34 + reinfusion. Prompt hematological recovery was observed in all pts (PMN > 500/cmm after 8–10 days, PLT > 20.000/

cmm after 11–18 days). Adverse events during HDCT included oral mucositis (two WHO grade 1, one grade 2), one reaction to cryoprotectant DMSO; one grade 3 cellulitis, one *S. epidermidis* sepsis, and one colitis due to *C. difficile.* HIV plasma viral load remained undetectable in 3 of 4 pts on HAART. Opportunistic infections were diagnosed during follow-up in 3 pts after at least 5 months from HDCT. Five pts obtained complete response after 1 month. Among these pts, 2 had relapse at month 5 and 12, respectively, while three of them are disease free at month 5, 11, and 18.
Conclusions: These first observations suggest that HDCT can be both feasible and successful in selected pts with refractory or relapsing HIV-Ly. Such intervention was believed unfeasible in the pre-HAART era.

P846 Pravastatin and Fluvastatin as pharmacological treatment for hyperlipidaemia in HIV-infected patients receiving HAART

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Objectives: A wide spectrum of lipid metabolism abnormalities have recently been described in HIV-infected patients receiving a protease inhibitor-based highly active antiretroviral therapy. Aim of our work is to evaluate the role of pravastatin and fluvastatin in the management of HIV-associated hypertriglyceridaemia and hypercholesterolaemia. An open-label, randomized, prospective study about the efficacy and the safety of pravastatin and fluvastatin as pharmacologic treatment for protease inhibitor-related hyperlipidemia was performed.
Methods: Plasma lipid levels of 662 HIV-infected patients referred to our tertiary care centre and on protease inhibitor-based antiretroviral therapy since at least 12 months have been evaluated. All patients established with HIV viral load <50 copies/mL and presenting hypertriglyceridemia, with or without hypercholesterolemia and lipodystrophy, of at least 6-month duration and unresponsive to a hypolipidaemic diet and physical exercise, have been treated with fibrates or statins for 12 months.
Results: One hundred and twenty of the 662 observed patients (18.1%) received pharmacological therapy, while eight patients were excluded from evaluation due to early drop out. With regard to the 112 evaluable subjects, pravastatin was employed in 21 subjects and fluvastatin in 19. At the close of 1-year follow-up, pravastatin led to a reduction of 32.8% and 39.5% vs. baseline triglyceride and total cholesterol levels, respectively ($P < 0.001$). At the same time, fluvastatin obtained a reduction of 30.2% and 36.6% vs. baseline triglyceride and total cholesterol levels, respectively ($P < 0.001$). During these 12 months, the statins showed a favourable tolerability profile (with slight gastrointestinal symptoms reported in five patients only), plasma HIV viral load did not present any variation, and the mean CD4+ lymphocyte count increased as expected.
Conclusions: Pravastatin and fluvastatin revealed in our study a similar and statistically significant efficacy in the treatment of diet-resistant hyperlipidemia, with a more favourable effect on the hypercholesterolemia. However, further, enlarged studies seem necessary in order to establish the most appropriate guidelines for the management of dyslipidemia associated with highly active antiretroviral therapy.

P847 The management of accidental injuries with retrovirus infected instruments

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Background: About 1 million needlestick injuries are reported yearly in the USA in health care and research environments, with an estimated daily rate of 2400 and most often involve nurses and physicians. These incidents are observed in about 15% of surgical procedures. Accidental injuries usually occur at the depth of the subcutaneous layer, thus the lymphatic pathway is mainly involved. The seroconversion rate from percutaneous exposure to HIV is about 0.3%.
Objective: To investigate the possibility of preventing seroconversion by local intervention after simulated retrovirus inoculation thus enhancing the effectiveness of systemic treatment.
Methods: In the feline model, treatment of feline leukemia (an experimental model for HIV) after subcutaneous injury was studied. In the canine model subcutaneous (SQ), intralymphatic and intravenous injections of 200 nm radioactive particles equal in size to retroviral bodies were performed at the distal part of hind limb. Blood and lymph samples were collected at the groin and both pathways were compared using parameters such as flow rates, particle arrival time, concentration, and accumulation. Local measures such as tourniquet application and antiviral agent injection at the site of injury were used to prevent dissemination.
Results: After SQ introduction, 90% of the inoculum remains locally for a prolonged time, with gradual release into the circulation, thus justifying local treatment. Infiltration of the injury site with 0.2% povidone iodine prevented viremia after needlestick if applied immediately. After SQ injection, lymph carried 1000 times more particles than blood, but with much less speed. Application of a tourniquet above the site of injury reduced the spread of inoculum, especially by the lymph pathway while massage enhanced dissemination. Fast arrival of the particles in the blood after intralymphatic injection confirmed existence of functional lymphaticovenous communications at the peripheral level. Based on these findings operating room guidelines were established to prevent infection during suspected HIV cases.
Conclusions: There is a physiological basis for immediate local treatment after accidental injuries with retrovirus contaminated instruments. Massage or movement of the affected limb should be avoided. Tourniquet application and infiltration of the injury site with an antiviral agent slows dissemination, decreases the amount of spreading virus and makes systemic treatment more efficient.

Nosocomial bacteremia

P848 Nosocomial bacteremia among pediatric patients in Kosova

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Objective: To describe the epidemiology of nosocomial bacteremia among pediatric patients in Kosovo.
Design: Retrospective observational–descriptive study of bacteremia based on positive blood cultures. Standard definitions proposed by the Centers for Disease Control and Prevention for bacteremia were used. Blood samples were processed in the Department of Microbiology Institute of Public Health of Kosova, between June 1, 2000 and May 31, 2001.
Setting: Tertiary Health Care University Hospital with 2400 beds.
Participants: All newborns in Obstetrical Clinic ($N = 13469$) and all hospitalized children in Pediatric Clinic ($N = 4510$) were included in study.
Results: A total of 714 blood cultures were processed during study period. Totally, 34.7% of all blood extractions had significant growth. Gram-negative rods represented 91.7% of all isolates, whereas Gram-positive bacteria were 8.3%. Most commonly isolated microorganisms were: *Klebsiella pneumoniae* with 63.3%; *Citrobacter*, 11.1%; and *Streptococcus aureus*, 7.6%. Hospitaly acquired were 77% of registered bloodstream infections. Resistant strains of *Klebsiella* species to cephalosporins were above 90%, except ceftriaxone 37.3%. The most common prescribed antibiotic was ceftriaxone received by 206 patients (52.4%). The median length of hospital stay from admission to infection was 15 days. Nosocomial bacteremia was the most frequent (38.4%) on children who stayed more than 20 days in the hospital. The crude mortality was 31.8%. First 5 days of staying were associated with highest mortality – 87.8% (36/41). 45.3% of patients with nosocomial bacteremia had respiratory tract diseases in admission in hospital. *Klebsiella* species were also most frequent microorganisms identified from death cases. 27.2% of patients did not receive adequate treatment.
Conclusions: Nosocomial bacteremia among most vulnerable population represents one of the main priorities of health care system in Kosova. Detailed investigation should be conducted for surveillance of main nosocomial infections and strategic projects of prevention of nosocomial infections and antibiotic policies should be implemented in the near future.

P849 *Stenotrophomonas maltophilia* pseudobacteremia in the accident and emergency department

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Objectives: *Stenotrophomonas maltophilia* was the sixth most common cause of bacteremia in our hospital in 2001. In 2000/1999 it ranked seventeenth as a cause of bacteremia. On examination of the patients' records, we found that in 2001/2002, 57% of these blood cultures (BC) were taken in the emergency department, compared with 29% in 2000/1999. The aim of this study was to investigate the apparent increase between the two time periods, and investigate why the apparent increase took place largely in our emergency department (A + E).
Methods: Isolates of *Stenotrophomonas maltophilia* from the emergency department were sent to the PHLS in Collindale, London for typing by Pulsed Field Gel Electrophoresis. Patients records were examined looking for any common factors among the patients and attending medical staff. Environmental screening was carried out. We investigated the blood culture technique in 27 A + E staff and compared this to the technique used by 27 ward staff. We cultured the contents of blood bottles used for ESR, U +E, Coag, serum, blood culture and blood group.
Results: All of the isolates typed by PFGE were the same, supporting our suspicion of pseudobacteremia. There were no common factors among the patients. Extensive environmental screening in A + E failed to show contamination with *Stenotrophomonas maltophilia*. Examination of blood culture technique in 27 A + E staff, showed that 25 staff members took the BC through an intravenous cannula along with a number of other bloods (FBC, U + E, glucose, ESR, Coag, etc.). 41% took the blood culture first or used a separate site. Examination of BC technique among 27 ward staff, showed that all 27 staff took the blood directly from the vein. 59% took blood for culture only, the remainder, took blood for FBC +/− U + E, at the same time. On the wards, blood for glucose, ESR, Coag, etc. was usually taken in the morning by the phlebotomist. We recovered *Stenotrophomonas maltophilia* from the culture of the ESR bottle contents. All other blood bottles were culture negative.
Conclusions: We conclude that this cluster of cases of *Stenotrophomonas maltophilia* pseudobacteremia was related to contaminated ESR bottles. Further discussion with colleagues in the PHLS revealed that *Stenotrophomonas* had been isolated from ESR bottles in the UK.

P850 Microorganisms isolated from neonatal bloodstream infections

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Objective: Analysis of etiological agents of bacteremia in Department of Neonatology Public Hospital no. 2 in 2002.
Methods: Neonatal Unit has 63 beds (with 8 NICU beds) and admitted 1620 patients in 2002. Blood cultures were incubated in BacT/Alert system (Bio-Merieux). Identification and susceptibility was performed by VITEK system (Bio-Merieux) and disk diffusion method.
Results: We studied positive blood cultures from neonates with sepsis (6), congenital infection (6), meningitis (1), preterm birth (9), osteomyelitis (1), soft tissue infection (1), seizures (1), congenital toxoplasmosis (1) and suspicion of infection (20). Fifty-nine (11.5%) from total 514 blood cultures obtained from neonates in 2002 were positive (63 isolates). The predominant pathogen was methicillin resistant *Staphylococcus epidermidis* (MRSE) – 35 isolates (55%). There were only 6 isolates (10%) of methicillin susceptible *S. epidermidis* and one *S. aureus* MSSA. Enterobacteriaceae were isolated in 7 cultures: six *K. pneumoniae* (10%) and one mixed culture of *K. pneumoniae* and *Enterobacter aerogenes* – all ESBL positive. From other cultures we isolated: *Enterococcus faecalis* – 4, *Streptococcus viridans* – 2, *Candida albicans* – 1, *Bacillus* sp. – 1, *Lactobacillus* sp. – 1 and *Corynebacterium* sp. – 1. We cultured 3 isolates of the genus *Hemophilus*: one *H. influenzae* and two *H. parainfluenzae* from patients with congenital infections. After analysis, 16 cultures (13 with *S. epidermidis* and 3 with other bacteria: *Bacillus* sp., *Corynebacterium* sp. and *Lactobacillus* sp.) were considered contamination.
Conclusions: Surprisingly we did not isolated any primary agent of neonatal infections – *Streptococcus agalactiae* and *Escherichia coli*. It could be explained by early profilaxis with amoxicillin-clavulanic acid. The dominating pathogen was *S. epidermidis* MRSE responsible mainly for catheter related infections and multiresistant *K. pneumoniae*. We can conclude that in Neonatology Unit dominated nosocomial bloodstream infections.

P851 First report on nosocomial blood-stream infection caused by *Burkholderia stabilis*

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Objective: Analysis of the significance of *Burkholderia stabilis* (*B. cepacia* genomovar IV) in nosocomial blood stream infections.
Methods: Blood culture system Bactec 9050 (Becton Dickinson) was used. Strains were grown on *Burkholderia cepacia* selective agar and identified by PCR with species specific oligonucleotides using strain suspensions as template. MICs were determined by agar-dilution technique according to NCCLS guidelines.
Results: Between July and October 2002 *B. stabilis* was isolated from hemocultures of seven patients presenting signs and symptoms of septicemia in the ICU of the Mersin University Hospital, Mersin, Turkey. All patients died. *B. stabilis* was isolated from blood cultures between 1 and 8 days prior to death. Four patients had one, two patients two and one patient four *B. stabilis* positive blood cultures. The strains showed identical pattern of resistance (resistant to amoxicillin, amoxicillin/clavulanate, cefuroxime, cefoxitin, aztreonam, gentamicin, cotrimoxazole; intermediate to cefotaxime, tobramycin, sensitive to piperacillin, ceftazidime, meropenem, imipenem, ciprofloxacin). *B. stabilis* was cultured from washing fluid of bronchoscope, bronchoalveolar lavage fluid, and water distillator and may have been transferred from there into patients.
Conclusion: Our data indicate that *B. stabilis* may cause nosocomial blood stream infections and should be regarded as an opportunistic pathogen.

P852 Evaluation of blood-stream infections in a tertiary care hospital in Turkey

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Objective: To evaluate the epidemiology and risk factors of bloodstream infections (BSI), to determine the mortality associated BSIs and identify independent predictors of mortality.
Methods: All patients with positive blood cultures followed at ANERH from November 2001 to April 2002 were included to the study and followed up prospectively.
Results: 308 patients with BSI were enrolled to the study. Mean age was 48.8 ± 21.1 years, and 189 (61.4%) were male. During study period 407 episodes occurred, of which 49.9% were nosocomial bacteremia, 23.8% were community acquired bacteremia, 22.6% represented contamination and 3.7% represented transient bacteremia. Gram positive microorganisms were the most commonly isolated microorganisms (68.1%). Nosocomial BSI occurred in 129 (42%) patients. The most common pathogens in nosocomial BSI group were coagulase negative *Staphylococcus* (28.3%), *S. aureus* (24.5%), *E. coli* (8.9%), *Acinetobacter* spp. (8.4%), *P. aeruginosa* (6.6%) and *Enterococcus* spp. (6.1%). Methicillin resistance was detected in 78% of staphylococcus isolates in nosocomial BSI group. 22.7% of the patients died. Mortality rate associated with bacteremia obtained as 17.8%. Mortality rate in nosocomial BSI group obtained as 36.4%. Multivariate analysis revealed age >60 years, nosocomial acquisition, hospital stay more than 7 days, stay in intensive care unit as factors associated with mortality rate.
Conclusion: It is possible to eliminate factors influencing the outcome of bacteremia especially in nosocomial bacteremia. The prevention of nosocomial infections and appropriate antibiotic treatment can improve the prognosis of patients.

P853 Pseudobacteremia caused by contaminated erythrocyte sedimentation rate tubes: investigation of an outbreak

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Objectives: Contamination of blood culture bottles may occur at any stage of inoculation and culturing. Pseudobacteremia can lead to unnecessary use of antibiotics, a prolonged admission period and wasting of valuable resources. Finding the source however, can be laborious and expensive. We investigated a prolonged outbreak of pseudobacteremia with nonfermentative Gram-negative rods in our hospital in order to find and eliminate the source.

Methods: The study design was case-control. All patients with contaminated blood cultures during the year 2000 were included. Species identification was performed using API 20NE (BioMerieux) and Phoenix automated microbiology system (Becton Dickinson). Controls were randomly selected from a group of patients with a negative blood culture, matched by date of sampling. SPSS software was used for analysis. Data related to patients and sampling technique were recorded. The liquid content of several commercial blood collection tubes was cultured.
Results: Forty patients and 40 controls were included. Sex and age were equally distributed. Cases were more likely to be admitted with fever (OR 3.61). Contaminated blood cultures were taken more often at the ER (OR 8.5), by a nurse (OR 8.3) in combination with hematological tests (OR 69.9). Blood cultures of cases were less likely to be drawn by trained laboratory staff (OR 0.1). Evaluation of the procedures showed that ER nurses lacked proper instructions and took blood cultures after filling the Vacutainer collection tubes, using the same device. Laboratory technicians used written instructions and knew the correct order when collecting blood for culture in order to avoid contamination. From the liquid content of the erythrocyte sedimentation rate (ESR) tubes and from the blood cultures biochemically identical strains were identified.
Conclusion: ESR tubes were the source of an outbreak of pseudobacteremia in our hospital. Blood cultures were most likely infected by the Vacutainer system needle after it was contaminated by the non-sterile liquid content of the ESR tubes. After the implementation of a protocol for drawing blood at the ER, the problem was reduced considerably.

P854 Nosocomial bacteremia in a community hospital: a 20-year prospective study

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Objectives: To evaluate the characteristics and prognostic factors of nosocomial bacteremia (NB) in adult patients.
Methods: During a 20-year period (1983–2002) all episodes of NB were studied in a 320-bed community hospital. Clinical and laboratory features of survivors and nonsurvivors were compared using the SPSS statistical packet.
Results: 8 episodes of NB occurred in 687 patients (57% males, 43% females) aged 65 ± 18 year. The length of the hospital stay until the NB was 19 ± 16 days. Malignancy (30%), diabetes (24%), chronic renal failure (13%), and liver cirrhosis (9%) were the main underlying conditions. These were rapidly or ultimately fatal in 43%. The most frequent sources of NB were: intravascular catheters (23%), urinary tract (21%), intra-abdominal (14%), respiratory tract (12%), skin and soft tissues (9%). The most frequent isolates were: *Escherichia coli* (165), *Streptococcus aureus* (110), coagulase-negative staphylococci (99), *Pseudomonas* spp. (94), *Klebsiella* spp. (53), *Enterococcus* spp. (49), *Bacteroides* spp. (47), *Proteus* spp. (30), and *S. pneumoniae* (29). Crude mortality was 30% and mortality related to bacteremia was 20%. In the multivariate analysis 9 variables were identified as independently associated with mortality: septic shock (OR: 4.9; 95% CI: 3.0–8.0), rapidly fatal underlying disease (OR: 6.4; 95% CI: 2.3–17.4), axillary temperature $<37^{\circ}$C (OR: 6.1; 95% CI: 2.1–16.0), liver cirrhosis (OR: 2.8; 95% CI: 1.4–5.3), age >65 years (OR: 2.3; 95% CI: 1.4–3.5), inappropriate antibiotic therapy (OR: 2.2; 95% CI: 1.3–3.4), previous corticosteroid therapy (OR: 2.2; 95% CI: 1.3–3.5), respiratory, gastrointestinal or unknown origin (OR: 2.2; 95% CI: 1.4–3.2), and infection due to a high risk organism (*Bacteroides*, *Pseudomonas* or *S. aureus*) (OR: 1.6; 95% CI: 1.1–2.4). While in the first decade of the study the urinary tract was the main source of NB and *E. coli* was the predominant microorganism, in the second decade the intravascular catheters constituted the main source and coagulase-negative staphylococci were the predominant microorganisms.
Conclusions:
1. The main sources of NB were: intravascular catheters, the urinary tract and intra-abdominal.
2. *E. coli*, *S. aureus*, coagulase-negative staphylococci, and *Pseudomonas* spp. were the predominant causative organisms.
3. Nine variables were significantly related with mortality.

P855 Analysis of clinical features, risk factors and outcomes of nosocomial bacteremia

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Objectives: The objective of this study was to determine clinical features, risk factors and outcomes of nosocomial bacteremia (NB).
Methods: 414 patients with bacteremia were investigated in our hospital. Patients who met the nosocomial infection definitions of CDC were included in this study. A chi-square test was used to compare categorical variables, Student-*t*-test and Mann–Whitney *U*-test were performed for analyzing continuous variables. Multivariate analysis was performed with logistic regression.
Results: 414 patients with 484 bacteremic episodes were included in this study. 277 (67%) patients survived and 137 patients (33%) died. 176 patients (42.5%) in intensive care units (ICU) (including emergency and surgery), 238 patients (57.5%) in non-ICU clinics were enrolled. Methicillin-resistant *Staphylococcus aureus* (MRSA) ($n = 105$, 21.7%), *Escherichia coli* ($n = 65$, 13.4%), *Pseudomonas aeruginosa* ($n = 60$, 12.4%), *Klebsiella pneumoniae* ($n = 59$, 12.2%) were the most common causative agents in NB. In univariate analysis, to be older than 55 years old ($\chi^2 = 15.47$, $P < 0.001$), hospitalization in ICU ($\chi^2 = 45.02$, $P < 0.001$), increased creatinin level (>1.4 mg/dL) (χ^2: 6.08, $P < 0.014$), requirement of inotropic drug on admission ($\chi^2 = 49.3$, $P < 0.001$) ($\chi^2 = 45.02$, $P < 0.001$), bacteremic episodes more than one bacteremia (χ^2: 34.34, $P = 0.002$), Gram-positive bacteremia ($\chi^2 = 8.23$, $P = 0.016$) were determined as risk factors for mortality in patients with NB. The mean values of AST, ALT, creatinin, leukocyte and hemoglobin level in patients with bacteremia who survived were found significant in relation with mortality ($P < 0.05$ for all variables). Multivariate analysis revealed that being older than 55 years old [Odds ratio (OR): 2.29, $P = 0.001$)], hospitalization in ICU (OR:3.38, $P < 0.001$), bacteremic episodes more than one bacteremia (OR: 4.05, $P < 0.001$), Gram-positive bacteremia (OR: 1.76, $P < 0.026$) were independent risk factors for mortality of NB.
Conclusions: MRSA and *E. coli* were the most common causative agents in NB. Advanced age, hospitalization in ICU and bacteremic episodes more than one bacteremia and Gram-positive bacteremia were independent risk factors for mortality of NB.

P856 Etiology of septicemia in hospitalized patients

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Objectives: Estimation of etiology of septicemia in patients hospitalized in a tertiary care hospital from VII 1999 to XII 2001.
Methods: The study comprised 127 patients with clinically diagnosed hospital- or community-acquired septicemia. Blood cultures were processed in a computerized BacT/Alert system (Organon Teknika). The strains were cultured according to standard bacteriological procedures and identified with the use of biochemical API tests (BioMerieux). Susceptibility to antimicrobial agents was assessed by a disk-diffusion method, according to the NCCLS recommendations, and VITEK tests (BioMerieux).
Results: Most of the patients with hospital- or community-acquired septicemia were hospitalized in the hematology-oncology ward (82.3% and 17.7%, respectively). In total 135 bacterial isolates have been cultured, comprising 92 clinical strains. Sixty-two of them (67.4%) were isolated from cases of hospital-acquired septicemia. Among isolated bacteria dominated coagulase-negative staphylococci (CNS) – 23 strains, including 18 resistant to methicillin (MRCNS), *S. aureus* – 9 (including 4 MRSA) and *E. coli* – 9 strains. Thirty strains (32,6%) were cultured from patients with community-acquired sepsis. Etiology of these cases implicated 7 strains of CNS (including 5 MRCNS), 7 strains of *E. coli* and 4 strains of *S. aureus* (including 2 MRSA). Among fungi *C. tropicalis*, *C. lusitaniae* and *C. parapsilosis* were isolated from blood cultures of patients with septicemia.

Conclusion: Methicillin-resistant staphylococci may be etiological agents of both community- as well as hospital-acquired septicemia. There is an increase in frequency of isolation of *Candida* species other than *C. albicans*.

P857 The influence of inappropriate empirical antimicrobial treatment on the outcome of intensive care bacteremias

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Objectives: Inappropriate empirical antimicrobial treatment has been related to mortality in intensive care units, but this relationship depends on the local flora and the place where the study was done. The aims of this study were to determine the prevalence of inadequate empirical antimicrobial treatment and to know its influence on clinical outcomes in critical ill patients with bacteremia.
Material and methods: During a five and a half-year period, from 1996 to 2001, 215 patients admitted to a 12-bed general adult intensive care unit with significant bacteremia were evaluated. Inadequate antimicrobial treatment of bloodstream infection was defined as the microbiological documentation of infection that was not being effectively treated at the time the causative microorganism and its antibiotic susceptibility were known. Clinical and microbiological variables were studied. A multivariate analysis was performed to determine the influence of inappropriate treatment on mortality
Results: The prevalence of inappropriate empirical antimicrobial treatment was 26% ($n=56$). The proportion of nosocomial-acquired bacteremias was significantly higher in the group with inappropriate treatment (91%) than in the group with appropriate treatment (77%), $P=0.025$. None of the other clinical variables were significantly different between cases with inappropriate and appropriate treatment: Years of age (mean $\pm$ sd 63.4 ± 14.1 vs. 61.9 ± 16.3), sex (m/f 36/20 vs. 100/59), septic shock (30.3% vs. 26.4%), high risk focus (23% vs. 37.5%), ultimately fatal disease (26.7% vs. 32.7%) and high risk microorganisms (58.9% vs. 45.2%). The principle etiologies of cases with inappropriate treatment were: *Acinetobacter baumannii* ($n=19$), CNS ($n=16$), *Enterococcus* spp. ($n=5$), *Escherichia coli* ($n=4$), *Candida* spp. ($n=4$) and *Pseudomonas aeruginosa* ($n=3$). Related mortality of bacteremia was associated with septic shock (OR 3.28, 95% CI 1.71–6.30), but not with inappropriate treatment (OR 1.29, 95% CI 0.64–2.60).
Conclusions: Intensive care bacteremias are frequently treated with inappropriate empirical antibiotics. However, inappropriate empirical antibiotic treatment was not related to mortality, probably due to characteristics of the local organisms causing of bacteremia in our setting.

P858 Bacteremia in febrile elderly presenting to a hospital emergency department

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Objective: Determination of the prevalence of bacteremia in elderly presenting to hospital emergency department.
Patients and methods: We retrospectively study the records of eight hundred and twenty-three patients (823) aged 65–85 years presenting to our Emergency Department with temperature $\geq 38.5^{\circ}$C and without specific viral illnesses during the last 15 years. Bacteremia (defined as presence of pathogenic bacteria in a blood culture), white blood cell count (WCC), and final diagnosis based on clinical features and laboratory investigations.
Results: Bacteremia was identified in 28 of 823 patients (3.4%). Pathogens isolated were *Streptococcus pneumoniae* (19), *Neisseria meningitidis* (2) and *Klebsiella pneumoniae* (7). Increased WCC counts ($P<0.001$) and brief duration of fever ($P<0.001$) were associated with bacteremia. Nevertheless, clinical features, including high WCC counts ($\geq 20 \times 10^9$/L) had <10% predictive accuracy for bacteremia. Overall, final diagnoses in the 823 febrile elderly patients included nonspecific viral infections (25%), upper respiratory tract infections (4%), infectious gastroenteritis (9%), pneumonia (37%), and urinary tract infection (25%).
Conclusions: Most elderly patients presenting to a hospital emergency department with temperature $\geq 38.5^{\circ}$C without a clinical focus have a viral infection. However, 3–4% have occult bacteremia. Neither clinical features nor high WCC counts reliably identify these patients. As empiric antibiotics may contribute to increasing antibiotic resistance and have not been shown to prevent the rare complication of meningitis, we believe that close contact and regular review of these patients is preferable to empiric antibiotic therapy.

P859 Anaerobic bacteremias

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Objective: Compare the incidence and clinical characteristics of anaerobic bacteremias with the difference of 10 years, analyzing the number of bacteremias during 1986–87, and 1997.
Methods: During 1985–86 (2 years), and 1997 (1 year), there were studied all the anaerobic bacteremias (46 anaerobic bacteremias with clinical significance). There were collected clinical and analytical characteristics of all of them by the study group prospectively, with intention to analyze if incidence of anaerobic bacteremias is decreasing and to analyze the most common characteristics of them.
Results: In 1985–86 about a total of 512 bacteremias, 22 (2.2%) were anaerobic bacteremias and of them: 8 were polymicrobial, 13 were by *Bacteroides* sp. (the most frequent one), and one of them by *Fusobacterium* sp. In 1997 about a total of 472 bacteremias, 24 (2.4%), were anaerobic bacteremias and of them: 6 polymicrobial bacteremias, 14 were by *Bacteroides* sp. (the most frequent one), 1 by *Fusobacterium* sp., 2 by *Clostridium* sp., and one of them by *Flavobacterium meningosepticum*. About the evolution to death, in the first period 10 (45.4%) were deaths, and in the second one 9 (37.5%), died. About the empiric treatment received before knowing the results of blood hemocultures, in the first period 17 (77.2%), received correct empirical treatment. In the second one 20 (86.9%), received correct empirical treatment. About the age of the patients in 1986–87, 14 (63.6%), were older than 60 years, and in 1997, 18 (75%), were older than 60 years.
Acquisition: In 1986–87, 8 (36.3%), were extrahospitalary acquisition, in 1997, 15 (62.5%) were extrahospitalary acquisition.
Origin: In 1986–87, 12 (54.5%) had an abdominal focus, 5 (22.7%) had skin or surgery wound and 3 (3.63%), were of unknown origin. In 1997, 13 (54.16%), were of abdominal origin, 3 (12.5%) had a skin or surgery wound focus, and 3 (12.5%), were of unknown origin.
Conclusions: Although there is a low anaerobic bacteremia incidence this one hasn't diminished in 10 years. Anaerobic bacteremias have a high mortality index although the most part of empirical antibiotic treatments are correct. The most part of patients are over 60 years, the most frequent origin of bacteremias is abdominal, following by skin and wound surgery, and unknown focus (similar in both periods), and the most frequent bacteria is *Bacteroides* sp., and polymicrobial bacteremias.

P860 Infection control strategies to reduce nosocomial blood stream infections related to central venous access devices: the use of control charts to analyze outcomes

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Objectives: To assess and interpret the effectiveness of strategies designed to reduce nosocomial blood stream infections (BSIs), related to central venous access devices (CVADs), using process control charts.
Methods: BSI surveillance data routinely collected at Royal North Shore Hospital, a tertiary referral center, indicated CVADs as an increasingly significant source of BSIs. A multiple approach prevention program, January 2001–November 2002 included the development of an Infection Control policy on CVADs and a hospital wide education program to enhance the profile of the risks associated with the insertion and care of these devices. Surveillance methods and data analyzes were improved by using a database system, 'electronic Infection Control Analysis Technology' (eICAT), designed by Queensland Health. The need to limit the insertion of these devices to accredited staff was reinforced and hospital sites for insertion of these devices limited to Anesthetics, Radiology and Intensive Care Units. Two process control charts, the Shewart and the exponentially weighted moving average (EWMA) chart measured the effectiveness of the program. Both the Shewart, which shows large changes indicating poor implementation of policies or unusual variation, and the EWMA, important for detecting changes in average rates due to systems and process changes, have proved useful in analyzing outcomes.

Results: As random variation was present we used an upper 95% control limit which is 2 standard deviations above the mean. The monthly count of BSIs related to CVADs prior to the introduction of the program exceeded the upper control limit for both the Shewart (U95S = 12.1) and the EWMA (U95E = 8). Random variation has continued, however, the EWMA shows a consistent trend towards the mean (6.1) and the data appear to remain in statistical control. Our most recent analysis demonstrates the data to be below the mean in November 2002.
Conclusion: The process control charts have shown a decrease in the number of BSIs related to CVADs. We believe process control charts are a useful tool for measuring the effectiveness of prevention programs.

P861 Bacteremia in a new Greek Hospital: a 2-year analysis

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Objectives: The aim of this study was to analyze the epidemiological and microbiological characteristics of all positive blood cultures yielded in the microbiology laboratory of our new hospital, which was inaugurated in 2000.
Methods: Henry Dunant Hospital is a new 355-bed tertiary hospital. The periods (A) 01/11/00–31/10/01 (first year of full activity) and (B) 01/11/01–31/10/02 (second year) were studied retrospectively for all episodes registered as true bacteremia. The blood cultures were processed and cultured automatically with Bactec 9240 system.
Results: During period (A) 7346 patients were admitted to Henry Dunant Hospital and 135 cases of bacteremia (18.4 cases/1000 admissions) were registered. Coagulase-negative staphylococci were the commonest microorganisms detected (38.5%, [MRSE 78.8%]), followed by *Pseudomonas aeruginosa* (14.8%), *E. coli* (7.4%), *Enterococcus faecalis* (7.4%), *Staphylococcus aureus* (5.2% [MRSA 87.5%]), *Acinetobacter baumanii* (5.2%), yeast (4.4%), *Enterococcus faecium* (2.9%), *Proteus mirabilis* (2.9%), *Klebsiella* spp. (2.2%) and various organisms of low incidence. Polymicrobial bacteremia was detected in one patient. During period (B) 23 723 patients were admitted to the Hospital and 310 cases of bacteremia (13.1 cases/1000 admissions) were registered. Still coagulase-negative staphylococci were the most prevalent organisms (27.1%, [MRSE 85.7%]), *E. coli* (9.7%), yeast (8.4%), *Pseudomonas aeruginosa* (7.1%), *Enterococcus faecium* (5.2%,[GRE 7.0%]), *Klebsiella* spp. (5.2%), *Enterococcus faecalis* (4.8%), *Acinetobacter baumanii* (2.9%), *Enterobacter* spp. (2.6%), *Salmonella* spp. (1.3%) and various organisms of low incidence. Polymicrobial bacteremia was detected in six patients.
Conclusions: We conclude that despite the progressively increasing workload (number of patients' admissions, new departments' activity) the total percentage of bacteremia decreased during the second year. However, we must notice the increasing number of serious pathogens occurring during the second year such as GRE, yeasts, *Ralstonia pickettii*, *Stenotrophomonas maltophilia*, as well as the higher incidence of polymicrobial bacteremia. This occurrence should be attributed to the wider spectrum of clinical syndromes admitted to our hospital during the second year and to the expansion of certain departments such as Hematology, Oncology and ICU.

Serious systemic infections: meningitis, septicemia and endocarditis

P862 The first French outbreak due to *Neisseria meningitidis* B:15:P1,12

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Introduction: *Neisseria meningitidis* is an exclusive human pathogen responsible of bacteremia and meningitis. In developed countries, most of meningococcal diseases are sporadic and serogroups B, C and W135 represent the majority of the strains isolated, as illustrated by the French situation where more than one half of the meningococcal diseases are due to serogroup B and quite a third to serogroup C.
Results: From November 2000 to February 2002, 13 cases of meningococcal disease, either meningitis or meningococcemia, were recorded in the department of Indre-et-Loire, France. Eight of the 13 cases were due to *N. meningitis* B:15:P1,12. Molecular typing performed by restriction fragment length polymorphism of the genes pilA and pilD showed that the eight B:15:P1,12 strains harbored the same restriction patterns. Six patients were male and two females and were 14–28 years old. Epidemiological investigation revealed that all eight patients lived in the same district of the department, and that two of them were closely related. For seven patients, symptomatology was typical, but evolution was signed by septic metastasis for one patient and by neurological sequels for five patients; the outcome of the illness was favorable for these patients after antibiotic treatment. For one patient, overall clinical picture was unusual atypical; death occurred before the admission to the hospital and the *N. meningitidis* strain was isolated from culture of a meningeal biopsy.
Conclusions: This is the first time that *N. meningitis* B:15:P1,12 has been isolated in Indre-et-Loire or limitrophe regions, and also the first time that this serogroup is implicated in an outbreak in France. The strain has spread rapidly among the population because it was responsible of five cases over a short period of 3 weeks secondary to the first case. Besides, the outbreak has conduct to death in one case and in severe forms for the other cases with subsequent neurological sequelae. Finally, none of the patients had underlying immune defects that may confer a predisposition to invasive meningococcal or had taken an immunosuppressive therapy. These elements may suggest that this *N. meningitis* B:15:P1,12 strain may possess a high virulence.

P863 The changing epidemiology of severe infections caused by *Neisseria meningitidis* over the last 7 years

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Objectives: Serogroup B *Neisseria meningitidis* has been traditionally the most frequent cause of severe meningococcal disease in Spain. In 1996 there was a concern over an increase in the incidence of meningococcal infection in Madrid due to serogroup C, and a vaccination campaign was started in 1997. To assess the evolution of admissions caused by *N. meningitidis* severe infections, the evolution of the responsible serogroups and the antimicrobial susceptibility of the isolates, we reviewed the 101 cases of *N. meningitidis* which occurred at our institution from 1996 to 2002.
Methods: Ours is a large teaching institution (1800 beds) serving a population of 640 000 inhabitants. Isolates were identified by standard procedures and susceptibility testing was performed using the broth microdilution method with 5% lyzed horse blood. Beta-lactamase production was detected using the nitrocephin test. Serogroup, serotype and subtype were determined at the National Reference Laboratory for Meningococci in Spain.
Results: The evolution of new cases was as follows: 1996, 16 cases (0.32/1000 admissions); 1997, 22 cases (0.38/1000); 1998, 11 cases (0.19/1000); 1999, 12 cases (0.22/1000); 2000, 11 cases (0.19/1000); 2001, 12 cases (0.19/1000); 2002, 17 cases (0.28/1000). The evolution of serogroups C/B (percentages) from 1996 to 2002 was: 87/13, 82/18, 45/55, 50/50, 36/64, 83/17, and 12/88, respectively. The incidence of severe infections due to serogroup C in children over the period of study was: 85% (6 cases), 86% (12 cases), 17% (1 case), 40% (2 cases), 36% (4 cases), 100% (2 cases), and 0% (0 cases). The evolution of isolates non-susceptible to penicillin (MIC 0.12–0.25 mg/L) from 1996 to 2002 was: 62%, 86%, 91%, 58%, 82%, 58%, and 70%, respectively. None of the isolates produced beta-lactamase. All isolates were fully susceptible to cefotaxime, rifampin and ciprofloxacin.
Conclusions: Our data show a decrease of severe infections caused by serogroup C *N. meningitidis* that paralleled the vaccination campaign, a changing epidemiology of serogroups over the period of study and a high incidence of penicillin-resistant (non-susceptible) isolates.

P864 Acute bacterial meningitis in adults. A review of 31 recent episodes in an Italian hospital

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Objectives: Bacterial meningitis has to be reviewed periodically because the specific microorganisms responsible for the infection vary with time, geography and patient age. Aim of this study was to evaluate clinical and laboratory findings of acute bacterial meningitis in adults.
Methods: The charts of all patients 19 years of age or older in whom acute bacterial meningitis was diagnosed at the Infectious Diseases Division of the Hospital of Lucca during the 1998–2001 period were reviewed. Patients with HIV-related immunodeficiency or tuberculosis infection were excluded from this study.
Results: During the 4-year period 31 adults (16 males and 15 females, median age 60 year, range 19–88) were treated for acute bacterial meningitis all of which were community acquired and 17 (54.8%) occurred in elderly patients (>65 years). Predisposing factors were present in 13 (41.9%) of the episodes. Nineteen (61.3%) pts had the classical triad of fever, nuchal rigidity and change in mental status. Seizures occurred in 1 (3.2%) pt. Cerebrospinal fluid pleocytosis with a neutrophilic predominance and hypoglycorrhachia and elevated protein levels were present in 90% of the pts. A pathogen was identified in 29 (93.5%) of the cases. The causative pathogens were *Streptococcus pneumoniae* 14 (all strains fully sensitive to penicillin), *Neisseria meningitidis* 5, *Hemophilus influenzae* 2, *Staphylococcus aureus* 2, *Listeria monocytogenes* 2, *Staphylococcus capitis* 1, *Leptospira* 1, *Enterococcus faecium* 1, *Bacteroides* spp. 1. Bacterial antigen was found to be of acceptable sensitivity: 72.7% in all evaluable pts. The overall in–hospital mortality was 13.3%. Two out of 4 fatality cases of acute bacterial meningitis were caused by *Staphylococcus capitis* and *Bacteroides* spp., respectively.
Conclusions: The causative organisms of community acquired meningitis in adults are mainly *S. pneumoniae* and *N. meningitidis*. In vitro resistance of *S. pneumoniae* to penicillin was not observed in this series. A substantial proportion of cases due to staphylococcal microorganisms and *Listeria monocytogenes* was observed in our geographic area.

P865 Acute bacterial meningitis in adults: a study of 186 cases in South-eastern Anatolia of Turkey

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Objective: The aim of study was to assess the epidemiology, diagnosis, clinic, and laboratory of the patients with acute bacterial meningitis (ABM).
Methods: This is a retrospective study that described all the cases of ABM was diagnosed in the Dicle University Hospital from June 1996 to December 2002. In 7-year period, 186 adult patients were diagnosed and treated. The diagnosis of ABM was established on cytochemical characteristic of CSF and positive Gram stain and/or CSF blood cultures.
Results: The mean age of the patients, 110 male, 76 female, was 30.2 ± 15.3 years (range 14–90). Otitis media 40(21.5%) and head trauma 12(6.5%) were identified as the main predisposing factors for ABM. Etiology was described only in 23 patients (12.4%). *Streptococcus pneumoniae* was the most common identified pathogen. Antibiotic treatment before admission was given to 61.8% of patients. On admission, symptoms of meningitis were predominant: 92.5% headache 88.2%, fever and 80.1% had nuchal rigidity, Twenty-nine patients (15.6%) died during hospitalization period. The significant risk factors for mortality were as follows: Elderly, comatose mental status, low CSF/blood glucose ratio, erythrocyte sedimentation rate and treatment of penicillin.
Conclusion: ABM still remains a serous infection. Early diagnosis and treatment may reduce fatal outcome and improve the course of the disease.

P866 Bacterial septicemia: susceptibility of contemporary blood isolates (2000–2002) from hospitalized patients in Europe, Canada and the USA to Ceftriaxone and other antibiotic compounds (TSN Database)

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Objectives: In this study we report susceptibility rates of ceftriaxone (CTX) and other relevant antimicrobials for key pathogens isolated from blood as reported to physicians by clinical microbiology laboratories in the USA, Europe (EU), and Canada.
Methods: We analyzed data (January 2000-October 2002) from The Surveillance Network® (TSN) Databases in France (Fr), Italy (It), Germany (Gy), Spain (Sp), Canada (Cn) and the USA to determine susceptibilities from routine test results as reported to physicians. Only inpatient data from isolates taken from blood specimen sources ($n = 394\,554$) were included in the analysis. Contemporary NCCLS breakpoints were used, except for Fr (CA-SFM).
Results: Coagulase-negative *Staphylococcus* spp., *S. aureus* (SA), *E. coli* (EC), *K. pneumoniae* (KP), *P. aeruginosa* (PA), *E. faecium* (EFM), *E. faecalis*, *S. pneumoniae* (SP), and viridans group streptococci (VGS), together comprised 80–84% of all isolates per country. Comparatively high incidence of EFM in the USA (4.5%) and It (2.2%) reflected high incidence of vancomycin resistant [R] strains in those countries (65.1% and 12.6%, respectively). Methicillin-resistant SA (MRSA) rates were USA 47.9%, It 40.5%, Fr 33.3%, Sp 30.5%, Gy 13.0% and Cn 15.4%. More than 98.5% of methicillin-susceptible SA (MSSA) (all countries) tested susceptible (S) to ceftriaxone (CTX). 89.2% (Gy) to 93.0% (Sp) of MSSA were ciprofloxacin S; 71.7% (USA) to 86.9% (It) were erythromycin S; 93.5% (It) to 99.2% (Fr) were gentamicin S. Among SP, penicillin resistance was 1.9% (Gy) to 17.2% (Sp). SP isolates were CTX-S 97.3% (0.8% R) USA, 99.7% S (0% R) Cn, 84.4% S (0.3% R) Fr, 100% S Gy, 97.2% S (1.1% R) It, 93.2% S (6.8% R) Sp. For all countries susceptibility of EC and KP was 100% to imipenem (IMI), >95% to amikacin, >90% to CTX. Putative extended spectrum beta-lactamase expression in EC remained rare comprising 0.7% in Gy to 3.2% in the USA. Among *H. influenzae* (HI), ampicillin R ranged from 5.0% in Gy to 30.0% in the USA. Among HI, CTX was >98.2% S in USA, Cn, and EU. Among PA, IMI-S ranged from 69.5% (It; 22.0% R) to 87.2% (Gy; 8.4% R), ceftazidime-S 61.7% (It; 28.3% R) to 86.9% (Gy; 7.7% R), and piperacillin-tazobactam-S 79.3% (Fr; 8.5% R) to 92.8% (Gy; 4.5% R).
Conclusions: Surveillance data can help guide empiric choice. Apart from VRE and MRSA, acquired resistance to parenteral beta-lactams and aminoglycosides remains low.

P867 No ESBL producing *Escherichia coli* strains were observed among 210 clinical blood isolates in Copenhagen, Denmark

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Objectives: Increasing resistance to antibiotics is a major problem in treatment of serious infectious diseases. Recently, extended spectrum beta-lactamases (ESBL) has attracted considerable attention. The aim of the present prospective study was to investigate the presence of ESBL in *E. coli* blood isolates during a period of six months at Herlev University Hospital in Copenhagen, Denmark. Furthermore, the isolates were studied for the prevalence of coupled- and multiresistance (≥4 different groups of antibiotics).
Methods: The disc diffusion method (Oxoid, Sweden) on agar plates was used. The isolates were tested for resistance to amoxicillin (amx), amoxicillin + clavulanic acid (amc), ceftazidime (cfz), cefotaxime (ctx), aztreonam (atm), sulphonamide (sul), trimethoprim (trm), nalidixic acid (nal) and gentamicin (gnt). Susceptibility to the antibiotics were defined using the breakpoints

according to SRGA/RAF. The MIC's were determined to cefuroxime and ampicillin as control by the agar dilution method.
Results: No ESBL producing strains were observed, since no resistance to amc, cfz, ctx and atm was detected. The frequency of resistant isolates was amx 41% (84/204 strains), sul 43% (86/202 strains), trm 23% (48/206 strains), nal 6.3% (13/206 strains), and gnt 2.5% (5/203 strains). Resistance to amx was coupled to sul (80%). Resistance to sul was coupled to amx (78%). Resistance to trm was coupled to amx (75%) and sul (90%). Resistance to nal was coupled to amx (69%), sul (85%), and trm (62%). Resistance to gnt was coupled to amx (100%), sul (80%), and trm (80%), however, only 5 resistant strains were isolated. Multiresistance were registered in 13 isolates (6.2%).
Conclusions: The present study indicates that ESBL is not a problem in clinical blood isolates in Denmark. Resistance to amoxicillin and sulphonamide was significant, and highly coupled. This correlates to the widespread use of these antibiotics in Denmark. Resistance to nalidixic acid and gentamicin is still low in Denmark.

P868 Study of bacteremias at the emergency department in a Spanish teaching hospital during 2002

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Objectives: To determine the microorganism most frequently isolated from blood cultures from the emergency department during 2002 in a University Hospital in Madrid and the in vitro susceptibility.
Methods: 1579 blood cultures were retrospectively studied during 2002 in patients attending the emergency department with a range of age from 15 to 104. Blood culture system used was BACTEC 9240 (Becton-Dickinson). Bacteremia was defined as the isolation of Gram-positive cocci or Gram-positive rods in two or more extractions and the rest of microorganisms in at least one extraction. Identification and susceptibility was performed by conventional technology and an automatic system (MicroScan, Dade Behring).
Results: From the total of blood cultures, 281 bacteremias were identified (17.80%). The microorganisms most commonly isolated causing bacteremia were *Escherichia coli* in 98 cases (34.87% of the total because 10 of them were included in the polymicrobial blood cultures), coagulase-negative staphylococci in 44 (15.66%), polymicrobial in 26 (9.25%), *Streptococcus pneumoniae* in 21 (7.47%) and *Staphylococcus aureus* in 11 cases (3.91%; 9 were MSSA and 2 were MRSA). The rest of bacteremias (93) were divided as follows; 28 *Streptococcus* spp. (9.96%), 24 enteric Gram-negative rods (8.54%), 19 *Staphylococcus* spp. (6.41%), 10 anaerobics (3.56%), 8 non-fermentative Gram-negative rods (2.85%) and 2 yeasts (0.71%). The susceptibility of *E. coli* strains was as follows; 35.71% of the strains were susceptible to amoxicilin, 93.88% to amoxicilin-clavulanic acid, 98.98% to cefotaxime (the resistance was due to an ESBL production), 100% to imipenem, 86.73% to ciprofloxacin, 67.35% to TMP/SMX, 95.92% to gentamicin and tobramycin and 100% to amikacin.
Conclusions: *Escherichia coli* was the most common cause of bacteremia, with the 34.87% of the cases, of the blood cultures reported from our emergency department. The susceptibility pattern of this *E. coli* was within the expected values.

P869 Optimization of empirical antibiotic selection in the emergency department for suspected Gram-negative bacteremia

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Objectives: To identify risk factors for resistance of Gram-negative bacteria to first line antibiotics commonly used in the emergency department (ED) setting.
Methods: We conducted a one-year prospective study of patients admitted through the ED, who had positive blood cultures. Baseline data were collected, pertaining age, residence, prior antibiotic treatment, prior hospitalization or medical procedures, diabetes, immunosuppression, presence of foreign bodies, as well as current clinical data including infectious diagnoses, pattern of microbial resistance, APACHE score and outcome. Univariate analysis and multivariate regression analysis were carried out.
Results: A total of 246 ED-admitted patients had positive blood cultures. The spectrum of bacteria included 131 (53%) Enterobacteriaceae, 86 (35%) Gram-positive cocci and 29 (12%) others. We sought to determine risk factors for resistance among the Enterobacteriaceae. Of 131 isolates of Enterobacteriaceae, 32 (24%) were resistant to gentamicin and 37 (28%) were resistant to ciprofloxacin. Significant risk factors for resistance to gentamicin by univariate analysis were: older age, nursing home residence, prolonged recent hospitalization, foreign body and bacterial type ($P < 0.05$–0.0001); only presence of a foreign body was significant by multivariate analysis ($P < 0.05$). Significant risk factors for resistance to ciprofloxacin by univariate analysis were: male gender, older age, recent antibiotic treatment (especially prior ciprofloxacin use), nursing home residence, prolonged recent admission, foreign body and bacterial type ($P < 0.05$–0.00005); of which male gender, nursing-home residence and presence of a foreign body were significant by multivariate analysis ($P < 0.01$–0.001).
Conclusions: There is significant resistance to ciprofloxacin (28%) and/or gentamicin (24%) among Enterobacteriaceae isolated from blood cultures from patients admitted through the ED. Identifying risk factors for resistance against these commonly prescribed antibiotics allows for optimal selection of empiric antibiotic treatment of suspected Gram-negative infections by tailoring this to the patients' profile presented here. This may lead to improved patient outcome and more optimal use of antibiotics.

P870 Incidence of invasive pneumococcal disease in two Italian regions

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Objectives: Estimating the incidence of invasive pneumococcal diseases (IPD) is crucial to correctly design appropriate control strategies against these diseases. Reported incidence rates vary widely across European countries and USA. Determinants for the observed differences are unclear. So far no data on incidence rates of IPD were available for Italy.
Methods: A laboratory-based surveillance network was established, including all public microbiological laboratories (114), in 2 Italian regions (Piemonte and Puglia), respectively, with 4 289 731 and 4 086 608 inhabitants (14% of Italian population). IPD was defined as a case with isolation of *S. pneumoniae* from blood or CSF. Laboratory identified IPD in the two regions from April 2001 to March 2002 were prospectively reported and all isolates were sent to ISS (the Italian Public Health Institute) for further characterization. Moreover in 5 hospitals the local databases of hospital discharge records were retrospectively matched with the laboratory database, in order to determine the proportion of patients undergone to blood or CSF culture, as an explanatory variable for the observed incidence of IPD.
Results: Over the 12-month period of the prospective surveillance 129 IPD cases were identified in Piemonte and 24 cases in Puglia. The isolates from CSF were, respectively, 28 and 14. The incidence in children aged up to 23 months was 7.6*100 000 in Piemonte and 4.7*100 000 in Puglia. In the 65 years or older age-group, the incidence was, respectively, 5.7*100 000 and 0.2*100 000 in the two regions. The total number of blood cultures performed in Piemonte was 6 times higher than in Puglia (32.674 vs. 5007). In the retrospective study, in Piemonte a blood/CSF culture had been performed in 35% of patients with a diagnosis at discharge of pneumonia, in 40% of those with fever of unknown origin, and in 58% of those with meningitis. In Puglia these figures were 7%, 26% and 32%, respectively.
Conclusions: The active surveillance provided incidence rates different in the 2 regions. The population based incidence rates were lower than those reported in USA and some other European countries. The retrospective study showed a different attitude between the two regions in performing blood culture especially in pneumonia cases, which may represent a large number of IPD cases.

P872 Community-acquired bacteremias in critically ill patients

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Objectives: The goals of this study were to know the epidemiological, clinical and microbiological features of community-acquired bacteremias, and their clinical outcomes in critical ill patients, in a general intensive care unit.

Material and methods: Prospective observational study of 41 cases of community-acquired bacteremias in a 12-bed adult medical and surgical intensive care unit in a teaching hospital, during a period of 5 and a half years, from 1996 to 2001. Community-acquired bacteremia was defined as the isolation of one or more organisms from blood cultures taken before 48 h after admission, which were not related to an invasive procedure. Epidemiological, clinical and microbiological features were recorded from clinical charts. To determine the clinical outcomes global and related mortality were evaluated and compared with clinical outcomes of nosocomial bacteremias by Chi-square test.
Results: Forty-one (19%) from a global of 215 clinical significant bacteremias in critical ill patients were community-acquired. The mean age of patients was 56.2 years ± 17.8, and the distribution by sex was 49% men and 51% women, with the following distribution of focus of infection: respiratory 24.4%, urinary tract 14.6%, abdominal 14.6%, endocarditis (7.3%), unknown 34.1%, and others 4.9%. Thirty-six percent of patients had an pre-existing comorbidity classified as ultimately or quickly fatal disease according with MacCabe classification. The most frequently causative organisms of community-acquired bacteremia were *Staphylococcus aureus* ($n = 11$), *Escherichia coli* ($n = 10$), *Streptococcus pneumoniae* ($n = 6$) and *Pseudomonas aeruginosa* ($n = 4$). The frequency of severe sepsis and septic shock were 73.2% and 48.8%, respectively. Inappropriate empirical treatment was given in 12.2% of cases. Global mortality was lower in community-acquired bacteremias (39%) than in nosocomial bacteremias (56.3%), $P = 0.046$, but related mortality was great in both groups, 29.3% and 25.3%, respectively ($P = 0.601$).
Conclusions: Community-acquired bacteremias in critical ill patients had a high global and related mortality, Classic pathogens such as *Staphylococcus aureus* and *Escherichia coli* were the most frequently organisms isolated, but *Pseudomonas aeruginosa* should be considered as an etiological agent of community-acquired bacteremias.

P873 The changing epidemiology of *Staphylococcus aureus* bacteremia: a 10-year study in a community hospital

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Objectives: To determine the episodes of significant *Staphylococcus aureus* bacteremia in a community hospital over a 10-year period, and to evaluate methicillin-resistance of the strains (MRSA) and the origin (nosocomial or community-acquired) of the bloodstream infections.
Methods: During a 10-year period (1993–2002) we have evaluated all *S. aureus* bacteremia recovered from patients admitted to the community-teaching hospital. Antibiotic-susceptibility of the strains by several methods (disk diffusion assay, ETest-MICs, latex agglutination test-MRSA-Screen) and mecA PCR-analysis for detecting methicillin-resistance were performed to estimate the proportion of nosocomial or community-acquired MRSA during the study period. Sensibility to glycopeptides and linezolid from all strains were also tested.
Results: A total of 306 significant *S. aureus* bacteremia cases were detected, with a progressive increase over the years, from which the 19.6% (60 cases) were MRSA. Considerable differences were observed when the distribution of MRSA isolates of different origin (community or nosocomial) and period of the study were compared. At the first time (1993–96), with only the 19.3% (59) of the bacteremia cases, ICU-MRSA outbreak grouped the 57.9% of the 32.2% MRSA detected in this period. The second one (1997–99) exposed the 36.2% of the cases (111), with a MRSA decrease to 12.6% located in the ICU and internal ward. However, in the last period of the study (2000–2002), that grouped nearly 50% of the cases, MRSA increased until 27.8% in 2002. In difference to the two previous periods, the origin of MRSA cases were mainly community-acquired (44.4%), whereas no MRSA cases in ICU-surgical wards were detected. All MRSA isolates were glycopeptides (vancomycin, teicoplanin) and linezolid 'in vitro' sensible.
Conclusions: The prevalence and origin of MRSA bacteremia cases changed during the study. The initial problem with MRSA nosocomial-ICU-outbreak was resolved in time, emerging in the last 3 year period the community-acquired cases, that seems to increase substantially.

P874 Mortality associated with community-onset *Staphylococcus aureus* bacteremia

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Objectives: *Staphylococcus aureus* bacteremia (SAB) is still a serious and common health problem. Data concerning Community-Onset SAB (COSAB) are scant, so that we performed a study to describe the mortality of COSAB and to analyze the factors associated with it.
Methods: Retrospective analysis of all 73 patients with a positive blood culture for *S. aureus* within 48 h after hospital admission (March 1998–October 2001). Patients on hemodialysis, or regular visits to day hospital were excluded. Demographic and microbiologic factors, foci of infection, underlying conditions, previous hospitalization, preadmission antibiotic use and outcome were evaluated. Results [percentages, median (range)] were compared by the chi square or Student *t*-test as appropriate. A logistic regression (LR) model evaluated the factors independently associated with mortality.
Results: Median age was 73 years (0–99), 52% were female and 30% were nursing home residents. Twenty-two percent of *S. aureus* strains were resistant to methicillin and 81% of the patients had an apparent focus of infection. Median length of hospital stay was 10 days (0–82) and 30% of the patients died. By univariate analysis, death was associated to be older (85 vs. 66 years), nursing home resident (55% vs. 20%), dementia (50% vs. 22%), no recent betalactamic treatment (36% vs. 0%), shorter hospital stay (8 vs. 11 days) (all $P < 0.05$), and no evident focus of infection (50% vs. 25%, $P = 0{,}10$) but not with sex, methicillin resistance, recent non-betalactamic antibiotic use, or previous hospitalization. By LR, nursing home institutionalization (OR 5,02; 95% CI 1.24–20.31), identified focus of infection (OR 0.21; 95% CI 0.05–0.94), preadmission antibiotic use (OR 0.14; 95% CI 0.02–0.96) and, marginally, age (OR 1.03; 95% CI 0.99–1.07), were associated with death. In contrast, methicillin resistance (OR 1.89; 95% CI 0.36–9.81) was not associated with death.
Conclusions: COSAB has a high mortality, especially in nursing home residents not recently treated with antibiotics with no apparent focus of infection. However methicillin resistance was not associated with death.

P875 Blood stream infections at an infectious diseases hospital in Ho Chi Minh City, Vietnam: 1993–2000

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Objective: To study the changing pattern of bacteremia and fungemia at an infectious diseases hospital in Ho Chi Minh City, Vietnam, between 1993 and 2000.
Methods: Retrospective analysis of hospital and laboratory records.
Results: There were 31 720 blood cultures taken in 29150 patient episodes. In 4292 (14.7%) episodes an organism was isolated that was considered significant. The proportion of positive episodes in children was significantly greater than in adults (1371/6677 (20.5%) vs. 2918/22 473 (13.0%), $P < 0.001$). *Salmonella* was the predominant isolate (3164 (73.8%) episodes) and *Salmonella typhi* accounted for 96.1% of these isolates. *S. typhi* isolations varied each year with peaks in 1995, when 90% of strains were multidrug resistant, and in 1998, when 76% were also nalidixic acid resistant. In 2000, 41% of *S. typhi* strains were multidrug-resistant and 47% were nalidixic acid resistant. *Salmonella paratyphi* A accounted for 2.9% of *Salmonella* isolates and were rarely drug resistant. *Escherichia coli*, *Klebsiella* and *Staphylococcus aureus* were the commonest other isolates. There was a significant decline in the susceptibility of *E. coli* and *Klebsiella* to gentamicin, ofloxacin and ceftriaxone, and *S. aureus* to methicillin over the study period. Isolations of *Cryptococus neoformans* and *Penicillium marneffei* increased, and this was related to the increasing number of patients admitted with HIV infection.
Conclusion: Important changes in the etiology and antimicrobial susceptibility of organisms causing blood stream infection were identified over the 8-year period.

P876 Epidemiology and clinical characteristics of patients with unsuspected endocarditis detected after elective valve replacement

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Background: The diagnosis of infective endocarditis (IE) is primarily based on clinical and laboratory criteria and may be confirmed by histology or cultures of the excised valves. The presence of only an inflammatory infiltrate in surgically removed valves is insufficient to fulfill present (Duke University) criteria for IE.
Objective: To determine the prevalence and significance of inflammatory changes in valves excised during operations for reasons other than IE.
Methods: The charts and histopathology of all patients undergoing valve replacement during eight years were reviewed in order to identify those with an unexpected valvular infiltrate. A total of 534 patients, 264 females and 270 males, mean age 64 ± 17, underwent 621 valve replacement(s) during this period; aortic valve (250, 47%), mitral valve (197, 37%), and both (87, 16%). Eleven of these patients (2%) underwent valve surgery during the course of diagnosed IE; these were excluded from this study. All excised valves were cultured and examined histologically for the presence of an inflammatory infiltrate, vegetations and microorganisms.
Results: The histopathologic examination of valves from 5 of 523 patients (1%) who had elective valve surgery for reasons other than IE, unexpectedly demonstrated inflammatory infiltrates suggestive of IE. There was no clinical or laboratory evidence of endocarditis prior to surgery in any of these patients. Blood and valve cultures, and serologic tests for *Mycoplasma*, *Chlamydia*, *Legionella*, Q fever, *Brucella*, *Rickettsiae* and *Bartonella* were negative in all, as was a thorough evaluation for antiphospholipin syndrome and thrombophilias. The patients were treated with antibiotics for culture-negative IE. Three patients had an uneventful recovery; one developed intramyocardial abscesses and expired during cardiac re-operation; one had recurrent fever and dehiscence of the aortic and mitral valve prostheses and after two cardiac re-operations is still in severe heart failure.
Conclusions: An inflammatory infiltrate in a valve excised for indications other than endocarditis may be present in as many as 1% of such operations. These infiltrates could be clinically significant and warrant empiric antimicrobial therapy.

P877 IVDA endocarditis in a tertiary hospital: a 22-year retrospective study

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Objectives: A study to document the characterization and evolution of infectious endocarditis in intravenous drug addicts (IVDA) in Prague, the capital of the Czech Republic.
Methods: A retrospective study on IVDA endocarditis patients who have been hospitalized in the Department of Infectious Diseases since 1980. The data of postdischarge condition of patients were collected with phone or personal contacting the patients, their families, or GPs.
Results: In years 1980–2002, 29 episodes of endocarditis in 26 intravenous drug addicts (14 men: 12 woman) were treated in our Department. The patients were 18–36 years old; the average age was 26 years. The etiology was *Staphylococcus aureus* in 22 episodes (76%), *Streptococcus mitior* (1×), and *Neisseria cinerea* (1×). Culture negative endocarditis due to previous antibiotic treatment was found in 5 episodes (17%). Vegetations were localized on the tricuspid valve in 21 patients (72%), on the aortic and/or mitral valve in 5 patients (21%), and on the both left-side and right-side valves in 2 patients (7%). Predisposing condition other than narcomania was found in 1 patients; it was a bicuspid aortic valve. Twenty-three episodes were cured. Relapses (>30 days and <1 years after stopping the antibiotic treatment) occurred in four of them. The relapses began 36 days till 3 months after stopping the antibiotic cure. Six patients (21%) died. The reasons were multiple organ failure (2×), lung failure (2×), congestive heart failure + brain embolism (1×), and massive lung embolism (1×).
Conclusions: The prevalence of IVDA endocarditis has been rising: There were 4 episodes in 1980–89, 2 episodes in 1990–94, 15 episodes in 1995–99, and 8 episodes in a 3-year period of 2000–2002. This trend correlates with the prevalence of narcomania in the Czech Republic.

P878 The comparison of 154 infective endocarditis cases for etiology, prognosis and treatment between two time periods (1992–2001 and 1978–92)

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Introduction: Infective endocarditis (IE) is a major cause of mortality and morbidity in Turkey which rheumatic hearth diseases is still a serious health problem, against new improved techniques and treatment protocols.
Methods: The microbiological and laboratory results with the clinical findings of 66 cases (42 male and 24 female), hospitalized and treated with the diagnosis of IE were investigated for the treatment and prognosis in Cardiology Institute between the years of 1992–2001 (II. period) for 10-year period in this study. The results were compared with the similar parameters of 88 cases monitored between the years of 1978–92 (I. period).
Results: Specifically decreasing rheumatic hearth disease ratios against the I. period was an important predispozone factor in the II. period with the ratio of 48%. The positivity of the blood cultures were detected as 53% and 54% in the I. and II. periods, respectively. *Staphylococcus epidermidis* was the common microorganism isolated as 15 and 10 cases in I. and II. period, respectively. The second isolated microorganism was *Staphylococcus aureus*, as 12 and 8 cases of I. and II. period, respectively. It was found a significant difference for the detection of prosthetic valve endocarditis in the II. period against the I. period. It was not found a significant relationship between causative pathogen and death but the death ratio was decreased from 28% to 12.5% in cases with *S. epidermidis* and decreased from 40% to 17% in cases with *S. aureus*.
Conclusions: As conclusion, in spite of a decrease in the last 10 years, acute rheumatic fever and rheumatic hearth diseases are still important health problems in Turkey. Besides that, prosthetic valve endocarditis cases were increased significantly when compared with the previous years. It was detected that there was no significant difference for the known causative patterns in both of the I. and II. period. It was concluded that, the mortality and morbidity of IE were decreased with increasing personal hygiene, improving medical approaches and new therapy methods.

Opportunist infections in immunocompromised hosts

P879 Opportunistic infections in patients treated with Infliximab: experience from a single institution (1999–2002)

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Introduction: Infliximab is a humanized monoclonal antibody against TNF-alpha approved for the treatment of selected patients with rheumatoid arthritis (RA), Crohn's disease (Cd) and others. TNF-alpha is a cytokine implicated in the initial immunologic response to infection.
Objective: To describe the epidemiology and risk factors for opportunistic infection in patients treated with infliximab in our institution.
Methods: Retrospective analysis of all patients treated with infliximab in our center between 1999 and 2002. Since 2001, PPD testing was routinely performed in all patients prior to initiation of infliximab. All PPD positive patients had a chest X-rays; treatment of latent disease was done with isoniazid during 9 months.
Results: Sixty-four patients had been exposed to infliximab (45 rheumatoid arthritis, 6 ankilosing spondiloarthropaty, and 13 with Crohn's disease). 47 were women, mean age was 45 years old, 52 in RA and 38 in Cd ($P = 0.02$). RA group received more infliximab dosage during more time than Cd group.

PPD tests were positive in four patients, all in AR group, that were treated with isoniazid. Another four patients (6.2%) developed opportunistic infection: three had tuberculosis and one visceral leishmaniasis. All tuberculosis were extrapulmonary and/or disseminated (two cases of miliary TB and one with gastrointestinal, hepatic and lung involvement). Previously treatment, PPD testing and chest-X-rays were negative in all cases of diagnosed TB. One patient had a paradoxical reaction 1 month after start of treatment. All patients responded to treatment. The patient that developed Kala Azar 4 months after infliximab therapy, had an excellent response to amphotericin lipidic complex therapy. No one in Cd group developed opportunistic infection.
Conclusions: Our data support an association between infliximab and opportunistic infections as previously reported. TB was the most frequent infection, presenting as a disseminated or extrapulmonary illness. Screening for TB infection is needed in this population before the start of infliximab therapy. Anergy status could be a problem due to basal immunodepression and TLIT should be considered in PPD-negative patients with known previous TB contact. To our knowledge, leishmaniasis has not been previously described in this setting.

P880 *Mycobacterium tuberculosis* in patients with cancer and marrow transplantation (1990–2000)

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Objective: *Mycobacterium tuberculosis* remains a serious cause of morbidity and infection-associated death worldwide. Patients with defects in cellular immune response have increased risk of reactivation and tuberculosis in this setting may lead to systemic dissemination. Characteristics of *M. tuberculosis* infection in susceptible patient population in a region of low endemicity were studied.
Methods: Retrospective analysis during 1990–2000 was performed in patients receiving care at The University of Texas, M.D. Anderson Cancer Center. All patients with HIV-I, and HIV-II infection were excluded.
Results: In 30 patients, 19 (63%) had hematological malignancy; head and neck involvement was most common ($n=4$; 36%) among solid-organ cancer. Eighteen (60%) were foreign-born, and 4 (13%) had received systemic corticosteriods. Fever was present in 97%, hemoptysis (30%), and night sweats (27%) were less common. In greater than half of pulmonary infections ($n=11$; 58%), lung lesions were initially mistaken for a neoplasm. No multidrug-resistant strain was identified, and in 20% ($n=7$ of 29) death was attributed to tuberculous infection, 57% ($n=4$) of these had received high-dose systemic corticosteroids ($P<0.001$).
Conclusions: Tuberculosis was uncommon in patients undergoing treatment for cancer, and mostly occurred in persons born outside of the US. Treatment with high-dose, systemic corticosteroids significantly increases mortality in cancer patients with *M. tuberculosis* infection.

P881 The prevalence rate of *Cryptosporidium* in patients receiving immunosuppressive drugs in Amircola Pediatrics Hospital, Iran, 2002

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Objective: Cryptosporidiasis is one of the problems in patients receiving immunosuppressive drugs. Considering the presence of broad range of clinical manifestations in such patients, following secondary infections, longevity of patients, the new methods of treatment and prevention, are continually in change. Hence, the aim of this study was to determine *cryptosporidium parvum* in 100 patients receiving immunosuppressive drugs considered as the test group and another 100 patients with gastroenteritis but nonimmunosuppressive drugs users, as the control group.
Methods: The patients under study were 100 for test and 100 for control, which were matched for sex, age, geographic conditions of the residing places (rural and urban areas). All the data concerning the patients such as age, sex, the kind of disease, the kind of drug used, the doses being used, contact with animals, the kind of drinking water, the place of living, duration of chemotherapy and the other clinical symptoms were obtained from the questionnaire. From the test and control groups, stool samples were collected thrice, and were stained by Ziehl–Neelsen and modified Ziehl–Neelsen methods. The results were analyzed statistically.
Results: The results of the study indicate that the rate of infection in test and control groups is 5 and 6%, respectively. The average infection rate was (5.5%). There was no significant difference in the rate of infection between test and control groups statistically ($Z=0.3$). The highest rate of infection was observed in the age groups of 4–6 years and 6–8 years in the test and control groups, respectively. There was no significant difference between sex and the rate of infection. The highest rate of infection was observed in patients with acute lymphatic leukemia, and the number of drugs used had no effect on the rate of infection.
Conclusion: The result of this study indicates that more accurate laboratory diagnostic study of this parasite in more individuals with normal and suppressed immune system is required.

P882 Evaluation of the etiology of infections in patients with hematological malignancies

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Objectives: To evaluate the incidence and distribution of infective agents in patients (pts) with hematological malignancies with primary Respiratory Syncitial viral (RSV) infection.
Methods: Retrospective analysis of all primary episodes (eps) of RSV infections at Hematological clinic was performed. Serological and PCR tests on viral pathogens and swabs from clinically suspect sites were taken. Additionally blood samples were collected and tested microbiologically.
Results: Fifteen eps of RSV infection were studied. All eps were diagnosed during the period of chemotherapy for hematological malignancies (3, chronic leukemia; 3, multiple myeloma; 6, acute leukemia). The RSV infection in four cases was diagnosed before eps of CMV and in one case before herpes virus 6. In these cases the treatment with IVIG 'Intraglobulin' (Biotest, Germany) plus aciclovir (GlaxoSmithKline, GB) reduced the clinical manifestations of disease. Bacteria were isolated from blood cultures in seven eps after RSV infection. These seven isolates were found: *Streptococcus pneumoniae* – four cases, *Staphylococcus aureus* – two cases and *Klebsiella pneumoniae* – one case.
Conclusion: Complex virological and microbiological control must be performed in eps of infections in pts with hematological malignancies in order to correct therapy regimen.

P883 Fever of unknown origin caused by primary CMV infection in a patient with Behcet syndrome

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Objectives: Fever of unknown origin (FUO) is a diagnostic challenge for the physician and needs both knowledge and experience. We could not find any report of CMV infection developing in a patient with Behçet syndrome (BS) and causing FUO (Medline 1966–2002). We reported such a patient presented with FUO and diagnosed as a primary CMV infection.
Case report: A 30-year-old-male was admitted with severe fever and shaking chills of 2 days. He reported a history of Behçet's disease of 2 years and use of azathioprine and cyclosporine A for recent 4 months. On examination, he appeared moderately ill. Temperature was 40.3 °C, pulse 120 beats/min, and blood pressure 100/60 mmHg. A mild, evanescent maculopapular rash was noted on face, neck, and dorsum. The remaining systems were normal. Hematocrit was 36%, WBC 3000/mm^3 (stab 8%, PNL 72%, and L 20%), plt 107 000/mm^3, ESR 44 mm/h, CRP 170 mg/L (N: <5). Azathioprine and cyclosporine treatment was discontinued. On follow-up, the fever continued, and the rash did not persist. An infectious focus could not be found. Leukocytes decreased to 1500/mm^3 and CRP level increased to 34 times of normal. He was as febrile neutropenic and initiated imipenem. Remitting fever persisted despite the therapy and CRP level did not decrease. He developed hepatosplenomegaly (3 cm each), and muscle pain. Liver enzymes were above three times of normal and bilirubin level was high (1.6 g/dL). Then WBC and plt counts increased to normal. The cultures remained sterile and the antibiotics were discontinued. Brucella tests, abdominal USG and CT revealed hepatosplenomegaly. Doppler USG of portal vein and lower extremities, echocardiography, ophthalmic examination, and chest X-ray were normal. The fever persisted for 4 weeks and efforts to find out the etiology lacked. Then his temperature seemed to decrease spontaneously. On 10th day, CMV-IgM was weakly (+) by ELISA, CMV-IgG being (–). CMV-68 kDa

antigen in leukocytes, CMV-DNA in both plasma and leukocytes were (+). CMV-IgM was measured two times (1 week apart) were (+). The corresponding CMV-IgG which was initially (–) measured as 2.4 ISR and 3.6 ISR ($N < 1.1$). At 30th day of the fever, CMV-DNA became (–). The temperature, liver enzymes and CRP levels normalized within 4 weeks. He was discharged as healthy and 3 months after, CMV-IgM was (–) and IgG was 4.6 ISR. In conclusion, CMV infection should be considered in patients with BS receiving immunosuppressive therapy.

P884 Focal lymphatic abscess due to *Salmonella* enteritidis

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A 45-year-old-type II diabetic woman was admitted to the hospital in November 2002 with right sided swelling and pain of the neck for 16 days. She had fever and difficulty in swallowing. The mass was first noticed 16 days prior to admission and became progressively larger and more tender. Except for the diffuse mass in the right submandibular region of about 60×50 mm in size, findings from physical examination were normal (i.e. without peripheral lymphadenopathy). Her medical history was not characteristic except for type II diabetes mellitus (unregulated) since 1998 and a history of dental abscess prior to the mass. Blood and urine cultures were sterile and stool culture revealed no pathogenic microorganisms on three separate occasions. An abscess specimen was processed using an automated monitoring system (BACTEC 9050; Becton, Dickinson and Company, Franklin Lakes, NJ), and it was positive for growth of a microorganism on the second day of incubation. Subcultures were plated on 5% sheep blood agar and eosin-methylene blue agar and incubated aerobically at 35 °C. After 24 h of incubation, Gram-negative bacillary microorganisms were seen at Gram stain of lactose negative colonies. Biochemical tests of the strain suggested that the microorganism was *Salmonella* spp. Subtyping according to Kauffmann–White using specific antisera (Denka-Seiken, Tokyo, Japan) defined that the isolate was *S. enteritidis*. Computed tomography revealed 60×50 mm conglomarate formed lymphadenopathy inferior to the right parotid adjacent to submandibular gland. An ultrasonography of the gallbladder was normal. Treatment consisted ciprofloxacin 2×2 g and metronidazole 4×1 g. Abscess was drained spontaneously on the 10th day of antimicrobial therapy. A review of the literature by medline found one previous report of focal suppurative lymphatic abscess due to *S. typhi*. In this case the first submandibular suppurative lymphatic abscess caused by *S. enteritidis* in a diabetic woman is reported.

P886 Prevalence of respiratory Syncytial virus infection in cancer patients

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Objectives: The purpose of this study was to determine in serum of cancer patients antibodies IgA and IgG against respiratory syncytial virus (RSV).
Methods: During December 2002 was collected sera from 80 cancer inpatients. Fifty of them had lung cancer (Group A) and the rest 30 (Group B) other types of cancer. All the sera were tested for detection of antibodies IgA and IgG against RSV by the micro-Elisa in plates method (Serion).
Results: Of the 80 cancer patients, 25 (31.2%) were positive for IgA RSV antibodies and 8 (10%) for IgG RSV antibodies. Of the 50 patients with lung cancer (Group A) 15 (30%) were positive for IgA RSV antibodies and 4 (8%) for IgG RSV antibodies. From Group B 10 patients (33.3%) were positive for IgA RSV antibodies and 4 (13.6%) were positive for IgG RSV antibodies.
Conclusions: Our study shows that the prevalence of RSV in cancer patients is low (10%) but the frequency of RSV active infection is intermediate (31.2%) during the winter months.

P887 Prevalence of Adenovirus infection in cancer patients

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Objectives: The purpose of this study was to determine in serum of cancer patients antibodies IgA and IgG against Adenovirus.
Methods: During December 2002 was collected sera from 80 cancer inpatients. Fifty of them had lung cancer (Group A) and the rest 30 (Group B) other types of cancer. All the sera were tested for detection of antibodies IgA and IgG against Adenovirus by the micro-Elisa in plates method (Serion).
Results: Of the 80 cancer patients were positive 72 (90%) for IgG Adenovirus antibodies as well as 44 (55%) for IgA Adenovirus antibodies and all of them were also positive for IgG Adenovirus antibodies. Of the 50 patients with lung cancer (Group A) 21 (42%) were positive for IgA Adenovirus antibodies and 43 (86%) for IgG Adenovirus antibodies. From Group B 23 patients (76.6%) were positive for IgA Adenovirus antibodies and 29 (96.6%) were positive for IgG Adenovirus antibodies.
Conclusions: This study showed that (1) there is a high Adenovirus infection prevalence (90%) and high frequency of Adenovirus active infection (55%). (2) There was not significant difference of adenovirus infection frequency between the two groups of cancer patients.

P888 Risk factor associated with coagulase negative *Staphylococcus* colonization of cancer patients

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Objective: To evaluate the prevalence of coagulase negative *Staphylococcus* (CNS) colonization in cancer patients with positive CNS blood culture, the most frequent site colonized and risk factor associated with CONS colonization in cancer patients.
Methods: To assess factors associated with CNS colonization, colonized patients were compared with noncolonized patients. Univariate statistical analyzes were conducted using the program EPIInfo 6.04 (CDC). Chi-square test and Student's *t*-test were used for categorical variables. For the quantitative variables the Kruskal–Wallis test was used. Odds Ratio (OR) and 95% confidence interval (95% CI) were calculated, the level of significance was $P < 0.05$. A multivariate model was built using the program Statistics/Data Analysis 7.0 (STATA) 2001. Variables which had a *P*-value of less than 0.1 in the univariate analysis were included in the multivariate model.
Results: Surveillance cultures (nasal swab, swab of skin on the site insertion of central venous catheter and retal swab) were performed in 60 cancer with a positive surveillance culture. Of these, 20 (34%) patients were classified as BSI, 14 (21%) as contaminants and 26 (45%) as possible BSI. Thirty-six patients were male, the age ranged from 27 to 81 years old (median of 59.5) and 31 (52%) were hospitalized. The most frequent underlying diseases was multiple myeloma (88%), 33 patients (69%) were submitted to bone marrow transplant. Mucositis was present in (38%), graft vs. host diseases in 2% and neutropenia in 50% of patients. Sixty-one percent had received levofloxacine prophylaxis and 75% were under systemic antibiotic before the collection of blood culture. Fifty patients (83%) had a positive surveillance culture to CNS. Nose was the site most frequently positive to CNS (62%), following by gastrointestinal (GI) tract (32%), and skin on the insertion site of CVC (30%). Mucositis grade II ($P = 0.0023$) was associated with colonization due CNS and neutropenia ($500/mm^3$) ($P = 0.004$) and severe neutropenia ($100/mm^3$) ($P = 0.006$) were associated with colonization of GI tract by the univariate analysis. However, only severe neutropenia remained significant in the multivariate analysis ($P = 0.0036$).
Conclusion: Our results suggest that cancer patients are usually nasal colonized by CNS and that severe neutropenia is associated with GI tract colonization by CNS.

P889 The effect of oral zinc supplementation on oropharyngeal infection agents in patients receiving radiotherapy and chemotherapy for head and neck cancer

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Objectives: Oral zinc supplementation is able to reduce the frequency of infectious diseases owing to opportunistic microorganisms. For this reason, we aimed to investigate the effect of oral zinc supplementation on oropharyngeal infection agents in the immunocompromised host.
Methods: Thirty patients receiving radiotherapy and chemotherapy due to head and neck cancer were included in this study. Patients were between 18 and 71 years old. They were divided into two groups: 15 patients were given zinc (150 mg/day = 3 Zinco 220 capsules per day) orally (treatment group), and other 15 received empty capsules as placebo (control group). All patients took the capsules everyday throughout the course of therapy and 6 more weeks. No patients received any antibiotic chemotherapy during the term of oral zinc supplementation. The oropharyngeal samples from the patients were collected four times: 1 day before the first course of radiotherapy and chemotherapy, 1 day after the last course of radiotherapy and chemotherapy, 1 and 6 weeks after the therapy. The samples obtained by swab technique were cultured and investigated for bacterial and fungal pathogens by using microbial diagnostic methods and the gas chromatography method (Microbial Identification System).
Results: Coagulase positive and negative staphylococci, group A beta hemolytic streptococci, *Streptococcus pneumonia*, and *Candida* spp. seemed as opportunistic infection agents in both groups according to the culture results. We found a decrease in amount of colonized *Candida* spp., coagulase negative and positive staphylococci in the treatment group in comparison with control group. But, any difference in amounts of group A beta hemolytic streptococci and *S. pneumonia* could not be seen between two groups.
Conclusions: However, oral zinc supplementation seems enough to prevent the opportunistic infections especially from *Candida* spp. and coagulase negative and positive staphylococci, we suggest low doses of antibiotics in accordance with the antibiotic susceptibility test results for these pathogens, along with oral zinc supplementation. Because any effect of oral zinc supplementation on group A beta hemolytic streptococci and *S. pneumonia* was not seen, it is essential to start the antimicrobial chemotherapy based on antibiotic susceptibility test results before radiotherapy and chemotherapy particularly in the presence of *S. pneumonia*.

P890 Oral cavity disinfection in prevention of stomatitis in patients with immunosuppression during systemic chemotherapy

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Introduction: Betadine solution is an iodide antiseptic with broad spectrum of antimicrobial activity. Application of this agent on skin or mucosa eliminates at least 85% microorganisms, which makes it one of the most effective antiseptics.
Objectives: Estimation of betadine activity on microorganisms colonizing nosopharynx of patients treated with chemotherapeutics. The main purpose of the test was to check the level of microorganisms elimination from the nosopharynx and to estimate of side-effects' intensity (mucosal complications after chemotherapy – WHO scale) in two groups.
Methods: In this work we compared two antiseptic agents in 31 patients (Betadine and hospital pharmacy prepared solution), which demonstrated leukopenia after chemotherapy. Those pts were treated in Hematological Ward. 15 pts had to gargle betadine three times a day and 16 pts were a control group (they used hospital pharmacy prepared solution). All of them had bacteriological examinations (nosopharyngeal swab and saliva) before they started using betadine and on 7th, 14th, 21st day of study. Samples were identified by standard methods and disc – diffusion susceptibility testing was done according to NCCLS.
Results: The patients were examined from March 2001 to February 2002. 364 bacteriological tests were done. They included 109 nasal swabs, 107 pharyngeal swabs and 158 saliva samples. We noticed presence of multisusceptible pathogens taken before the treatment in 15 cases, normal flora in 16 pts. There isolates were as followed: yeasts (49.3%), Gram-negative rods (37.7%), Gram-positive cocci (13%). Besides we cultured multiresistant *A. baumanni* and *S. maltophilia* in two cases (Table 1).

Table 1

Results	Betadine (%)	Control group (%)
Erradication	33	6
Failure	66	56
Changes of flora	0	37

We did not observe correlation between gargling with antiseptic and the number of microorganisms in nasal samples.
Conclusions: We conclude that Betadine gargled patients experienced faster pathogen eradication and faster mucosal recovery comparing to hospital pharmacy prepared solution.

P891 Application of *N*-chlorotaurine in rhinosinusitis complicating immunosuppression following heart transplantation

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Objectives: Severe infections are a frequent problem complicating immunosuppression following heart transplantation. *N*-chlorotaurine, a new endogenous antiseptic involved in innate immunity, is very well tolerated by human tissue and promising in local therapy of infections.
Methods: We report about a heart-transplanted, immunosuppressed patient suffering from bacterial sinusitis caused by *Pseudomonas aeruginosa*.
Results: In spite of repeated antibiotic therapy, septoplasty, conchotomy, and fenestration of the sphenoid sinus bacterial cultures remained positive and the infection persisted. It was finally cured by repeated irrigations using 1% *N*-chlorotaurine solution for 7 days.
Conclusions: The combination of functional endonasal sinus surgery and lavage with *N*-chlorotaurine proved to be an effective and well tolerated therapy in antibiotic-resistant sinusitis during immunosuppression.

P892 Chronic *Chlamydia pneumoniae* infection and risk of lung cancer

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Objective: To evaluate the association of past infection with *Chlamydia pneumoniae* (C.pn) with subsequent risk for development of lung cancer.
Methods: 80 cancer in-patients from Saints Anargyri Oncological Hospital (44 men and 36 woman, aged 40–65 years) with histologically confirmed invasive carcinoma of lung, were prospectively examined. 45 (56.25%) cases of cancer patients were small cell, 24 (30%) squamous cell, 5 (6,25%) adenocarcinoma and 6 (7.5%) large cell carcinoma. 100 healthy subjects (70 men and 30 women) aged 38–62 years were included as control group. In cancer patients and control cases 45% were smokers. In total 180 sera samples from cancer patients and healthy subjects were examined for the presence of IgG, IgA and IgM antibodies against C.pn. Specific antibodies against C.pn were performed by ELISA method.
Results: In patients group the presence of specific IgG, IgA and IgM antibodies against C.pn was 58.75, 18.75, and 0%, respectively. The percentages in control group were 30, 7, and 0%, respectively.
Conclusion: Higher levels IgG and IgA antibodies against C.pn were detected in cancer patients than in control group. In cancer patients 55-year-old-or younger who were smokers with small cell carcinoma or squamous cell carcinoma were diagnosed chronic C.pn infection more frequent. These results were indicated that chronic C.pn infection is possible to play a role in increasing the risk of lung cancer but the studies must continue with a larger number of cases.

P893 Serological evaluation of antibodies against herpes viruses CMV and EBV and *Helicobacter pylori* in liver cirrhosis

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Objective: The detection of IgG and IgM antibodies against herpes viruses CMV and EBV as well as IgG and IgA antibodies against *Helicobacter pylori* (H.P.) in cirrhotic patients.
Methods: 114 cirrhotic patients documented by biopsy were examined. 72 of them were men and 42 women aged 49–70 years. 100 healthy persons (70 men and 30 women) aged 45–68 years were included as control group. Specific IgG and IgM antibodies against CMV and EBV (VCA) as well as IgG and IgA antibodies against H.P. were performed by ELISA method.
Results: IgG seropositivity to CMV, EBV (VCA) and H.P. in cirrhotic patients was 92.98, 71.92, and 59.64%, respectively. IgM seropositivity to CMV and EBV (VCA) in patients group was 10.52 and 14.03%, respectively, while IgA seropositivity to H.P. was 19.29%. In control group, positive IgG antibodies against CMV, EBV (VCA) and H.P. were found 87, 70 and 43%, respectively. Positive IgM antibodies against CMV and EBV (VCA) weren't detected in control group cases while IgA antibodies to H.P. were detected in 11%.
Conclusions: (1) There isn't significant difference in IgG seropositivity to CMV and EBV (VCA) between cirrhotic patients and control group cases. On the contrary a significant difference appears for HP. (2) Various levels of IgM antibodies to CMV and EBV were detected in patients group. In control group weren't detected IgM antibodies. Higher levels IgA antibodies to H.P. were found in cirrhotic patients in relation with control group cases.

P894 Detection of IgG and IgM antibodies against herpes viruses CMV and EBV and parvo-virus B-19 in patients with beta-thalassemia major

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Objective: The occurrence rate of IgG and IgM antibodies against herpes viruses CMV and EBV and Parvovirus B-19 in polytransfused patients with beta-thalassemia major.
Methods: 76 patients with beta-thalassemia major were prospectively examined. 28 of them were men and 48 women aged 13–48 years. 100 healthy persons (70 men and 30 women) aged 20–45 years were included as control group. The detection of specific IgG and IgM antibodies against CMV, EBV and Parvovirus B-19 was performed by ELISA method.
Results: Positive IgG antibodies against CMV, EBV (VCA) and Parvovirus B-19 were found in 69 (90.78%), 59 (77.63%) and 49 (64.5%) thalassemia-patients, respectively, while in control cases positive IgG antibodies were found to CMV in 85 (85%), to EBV (VCA) in 70 (70%) and to Parvovirus in 65 (65%). Positive IgM antibodies to CMV in patients group were found in 3 (3.94%), to EBV (VCA) in 4 (5.26%) and none in patients against parvovirus B-19. Positive IgM antibodies to CMV, EBV and Parvovirus B-19 weren't found in anyone of the control group.
Conclusion: IgG seropositivity to CMV, EBV and Parvovirus B-19 in thalassemia patients group was similar with control group cases as well as IgM seropositivity to parvovirus B-19. IgM seropositivity to herpes viruses EBV and CMV seems to be significantly higher in patients with beta thalassemia major than in control group cases. These results are probably due to the large number of blood transfusion that this group of patients undergo and/or to immunosuppression of beta-thalassemia patients.

Clinical and epidemiologic aspects of HIV infection

P895 Evaluation of HIV-related nucleic acids and Papillomavirus DNA in cervicovaginal secretions of HIV-positive women

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Background: Human immunodeficiency virus (HIV)-seropositive women represent an important population at risk for acquiring cervical cancer and thus require frequent screening.
Objectives: To assess simultaneous HIV-related nucleic acids and human papillomavirus (HPV)-DNA in cervicovaginal secretions of a cohort of known HIV-seropositive women (no. 130).
Methods: Vaginal swabs were collected to diagnose infection by candida, trichomonas, or bacterial vaginosis. Proviral HIV-DNA, cell-associated and cell-free HIV-RNA in cervicovaginal secretions were analyzed using competitive polymerase chain reaction (PCR) and reverse transcription PCR. HPV types 6, 11, 16, 18, 31, 33, 35 and 56 were detected using PCR and subsequent restriction fragment length polymorphism analysis of PCR products.
Results: Proviral HIV-DNA, cell-associated and cell-free HIV-RNA were detected in 75 of 130 (58%), 51 of 130 (39%), and 45 of 130 (35%) lavage samples, respectively. 81 of 130 (62%) women had HPV-DNA in cervicovaginal secretions. The rate of HPV infection was related to severity of cytologically diagnosed squamous intraepithelial lesions. The rate of detection of HPV types of intermediate to high oncogenic risk was higher in HIV-positive women who tested positive for cell-associated or cell-free HIV-RNA in cervicovaginal secretions than negative subjects. Vaginal infection was more frequent among women who tested positive for cell-associated or cell-free HIV-RNA in cervicovaginal secretions compared with negative women.
Conclusions: The rate of HPV infection increases among women with high HIV viral loads in cervicovaginal secretions.

P897 A survey of the knowledge and preventive measures of HIV/AIDS in the New Juabeng District of the Eastern region of Ghana

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Objectives: HIV/AIDS pandemic has thrown a great challenge to the public health services of the world. Since the pandemic began, more than 60 million people have been infected with the virus. It is now the leading cause of death in the Sub-Saharan Africa. At the end of the year 2001, an estimated 40 million people globally were living with HIV/AIDS. The increase in HIV/AIDS cases, especially in the rural folks could be attributed to several social factors including formal education. Cross-sectional study was conducted in the New Juabeng District in the Eastern Region of Ghana to access the level of knowledge of the people on HIV/AIDS and its preventive measures in relation to their level of formal education and religious beliefs.
Methods: The survey instrument was an interview guide, which contained a mixture of open ended and close-ended questions. Respondents were interviewed after their house numbers had been randomly selected from a box.
Results: About 77% of the respondents were within the ages of 15–25 years representing the sexually active group in society. All respondents with secondary or tertiary education knew that, contact with infected needles and blades, unprotected sex and contact with infected blood were means of HIV transmission. About 97% of those with secondary education, 44% of those with junior secondary school education and 42% of those with primary or no formal education knew mother to child transmission of HIV. Significant population, that is, 12% of those with primary or no formal education knew no methods of HIV transmission whilst another significant population, that is, 4% of those with junior secondary school education did not know any method of HIV transmission.
Conclusion: All the people had heard of the disease HIV/AIDS but the level of formal education of the respondents markedly influenced an in-depth knowledge of the disease. Those with higher levels of formal education are

better informed. Religion also had an influence on the choice of preventive methods used by the respondents in HIV prevention. An improvement in the literacy levels of individuals as well as a modification of their religious beliefs would therefore greatly increase their level of knowledge on HIV/AIDS.

P899 Co-infection by hepatitis viruses in a monocentric cohort of 248 HIV-infected patients in a suburb of Paris during the era of HAART

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Objectives: To investigate the prevalence and characteristics of infections with hepatotropic viruses in a cohort of HIV-infected patients from a University Hospital in the suburb of Paris.
Methods: Two hundred and forty-eight consecutive HIV-infected patients (pts) attending the outpatients clinic (May–November 2001) were prospectively studied for socio-demographic, epidemiological, clinical and routine biological data. Anti-HAV and anti-HCV IgG antibodies (Ab), HBsAg and HbcAb were measured in all pts. HCV RNA and serotype ± genotype were determined in all HCV-positive pts and HBsAb in all HBcAb-positive pts; HbeAg, HbeAb and VHB-DNA in all HBsAg-positive pts and in case of isolate HBcAb. Anti-HDV IgG Ab and HDV-DNA were measured in all HBsAg-positive pts.
Results: The mean age of pts was 39.8 ± 9.7 years; 26% were females, 28% were intravenous drug users (IVDU) and 3% had received contaminated blood or blood products. Thirty percent were born in Sub-Saharan Africa. Thirty-five percent had declared AIDS. Eighty percent were ongoing current antiretrovirals; median CD4 and HIV-RNA were 345/mm^3 [2–2172] and 3750 copies/mL [<20–>500,000], respectively. The overall anti-HAV Ab prevalence was 79%: 84% in Sub-Saharan African pts, 66% in pts born in France and 73% in IVDU. The overall prevalence of HCV IgG was 35%: 96% in IVDU and 11% non-IVDU. Seventy-four percent of HCV Ab-positive pts had detectable HCV-RNA (55% type 1, 21% type 3, 10% type 4, 3% type 2, 10% untypable). Fifty-two percent of HCV-Ab positive pts undergone liver biopsy (20% had Metavir F4) but only 23% of them had been treated for HCV infection. Nine Sub-Saharan African pts were HCV positive (type 2, $n = 2$; type 4, $n = 2$; untypable $n = 2$). Twelve pts (5%) had positive AgHBs (five native from Sub-Saharan Africa) among whom two had positive AgHBe, three had positive DNA-HBV and two DNA-HDV. Nineteen percent of all pts had isolated positive HbcAb with no detectable DNA-HBV. Overall, 26% of pts had no marker of HBV infection and among HCV-RNA and/or AgHBS positive pts, 1/3 had no HAV IgG.
Conclusion: In our cohort of HIV-infected pts: (a) a high seroprevalence of HAV is observed, particularly in Sub-Saharan African and IVDU pts, (b) high type 1 or 4 HCV prevalence (65%) may predict a low response rate to antivirals, (c) immunization against HBV and HAV should be proposed to 26 and 33% of them, respectively.

P900 Late diagnosis of HIV infection in Portugal

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Introduction: HIV infection has a high incidence in Portugal. Diagnosis is nowadays easy, and information about transmission is well spread among population. Nevertheless, a high number of patients (pts) and doctors are still unaware of risk behavior, which leads to late diagnosis.
Objective: To evaluate the proportion of pts in whom the diagnosis of HIV infection and AIDS are coincident, and to compare two periods 10 years apart.
Patients and methods: Study of clinical records of HIV infected pts, observed in a major central hospital, between 2000–2002 and 1990–1992. HIV pts were included if the diagnosis of HIV infection and AIDS were coincident. Demographic data, risk behavior, AIDS defining disease and survival where recorded.
Results: From 2000 to 2002, 899 HIV infected pts were observed for the first time. In the 171 (19%) pts in whom AIDS was diagnosed at admission, age ranged from 14 to 67 years ($x = 38 \pm 13$ years); 143 (83%) were male. Risk behavior was IDU in 96 (56%), heterosexuality in 69 (40%) and bisexuality in 2; in 4 risk was unknown. AIDS was defined by tuberculosis in 78 (46%), PCP in 31 (18%), cryptococcosis in 14 (8%), esophageal candidiasis in 12 (7%), cerebral toxoplasmosis in 11 (6%), NH lymphoma in 6 (4%), KS in 5 (3%); other diagnosis in 14 pts. Death occurred in 64 (36%) pts, 0–835 days after diagnosis; 47 (73%) fatalities were in the first 6 months. In the tables we compare the two 3-year periods. (see Tables 1 and 2).

Table 1 Patients with AIDS and HIV infection diagnosis coincident

	Nr (%)	Male	Age, mean ± SD	IDU	Hetero	Homo/bis	Transfused	Unknown
1990–1992	50/287 (17)	39 (78%)	37 ± 10	13 (26%)	18 (36%)	12 (24%)	6 (12%)	1 (2%)
2000–2002	171/899 (19)	143 (84%)	38 ± 13	96 (56%)	69 (40%)	2 (1%)	–	4 (2%)

Table 2 AIDS defining disease

	TB	PPC	Cryptoc.	esof. cand.	Toxo	NHL	KS	6 months mortality
1990–1992	11 (22%)	14 (28%)	3 (6%)	3 (6%)	4 (8%)	1 (2%)	6 (12%)	39%
2000–2002	78 (46%)	31 (18%)	14 (8%)	12 (7%)	11 (6%)	6 (4%)	5 (3%)	73%

Conclusion: Late diagnosis of HIV infection is still common. Heterosexuals, not aware of the risk, do not perform screening tests, and IDU's are difficult to reach populations. Tuberculosis is common, according to the high prevalence of *M. tuberculosis* infection in Portugal. High early mortality is related to the late diagnosis. Strategies to reach these patients sooner are urged.

P901 Biologic characterization of a HIV-1 isolate involved in a primary infection associated with severe hemophagocytic syndrome

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Background: Symptomatic primary HIV infection is generally accompanied by more rapid clinical progression than asymptomatic infection. We performed biological characterization of an HIV-1 isolate involved in a primary infection, presented as severe hemophagocytic syndrome, and compared the properties of the newly transmitted virus to those of the donor virus.
Methods: Virus isolates were obtained by CD8-depleted coculture. Replication kinetics of the primary isolates were assessed by p24 release in supernatants. HIV-1 coreceptor usage was determined by growth on U87-CD4 cells expressing CCR5 or CXCR4. V3 loop region was amplified by nested RT-PCR. The amplicon underwent clonal sequence analysis, using Big Dye terminator method with ABI Prism 310 sequencer. Phylogenetic analysis was performed by the PHYLIP package software; inter- and intrapatient relationships were assessed by building a phylogenetic tree (neighbor-joining program).
Results: A 27-year-old-man was admitted to hospital with fever (40 °C), a diffuse nonpruritic morbilliform rash, generalized adenopathy and hepatosplenomegaly. Primary HIV infection with a life-threatening hemophagocytic syndrome was suggested by negative serology, presence of HIV p24 antigen, and high VL values. Clinical symptoms disappeared promptly after starting three drugs-based HAART and VL became undetectable. The asymptomatic viral source was identified and the virus was isolated from PBMC and plasma of both donor and recipient. The biologic characteristics of virus isolates from both donor and recipient were apparently indistinguishable for replication efficiency, coreceptor utilization (CCR5) and predicted phenotype (M-R5). Both isolates carried the I93L polymorphism of the protease gene. Molecular analysis showed the presence of a virtually homogeneous V3 quasispecies in the recipient, while highly divergent variants were found in the donor. The unique viral population present in the patient corresponded to a minor population of the donor quasispecies.
Conclusions: As frequently found during the early phases of HIV infection, circulating viral quasispecies were genetically homogeneous in the recipient, probably as the result of the transmission bottleneck. Biologic properties of the isolate, together with V3 region aminoacidic sequence, indicating M-R5 phenotype, did not account for the aggressive course of the infection, although it was promptly controlled by HAART.

P902 A case of fatal lactic acidosis triggered by amphotericin B in an AIDS-patient with visceral leishmaniasis

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Introduction: Although asymptomatic hyperlactatemia is common in HIV infected patients, lactic acidosis (LA) is rare and severe. The factors triggering the onset of LA are not yet well understood. A case of abrupt-onset and fatal LA coinciding with the start of amphotericin B (amphoB) treatment is reported. Thirty-four-year-old female ex-IV-drug user was diagnosed with AIDS (VL > 750,000 copies/mL; T4 4/mm^3), cryptococcal meningitis and chronic HCV and HBV in October 2000. She was lost to follow-up. In April 2001, pancytopenia (Hb 9 g/dL, leucocytes 900 and 56,000 platelets/mm^3) and bacterial pneumonia was diagnosed and HAART (AZT, 3TC and nelfinavir) was added to cotrimoxazol and fluconazol. VL became undetectable and T4 cells rose to 54 cells/mm^3. In May 2001 she was treated with amphoB 0.7 mg/kg per day for 2 weeks for relapsed cryptococcal meningitis without problems. Pancytopenia persisted. In September 2001 AZT was switched to d4T. In March 2002, visceral leishmaniasis with pancytopenia and massive bone marrow involvement was diagnosed. Treatment with amphoB (0.7 mg/kg per day) was started. Before start, anion gap, HCO_3 and CK's were normal, and LDH slightly elevated (1.3 times ULN). Lactate level 3 days before start of amphoB was normal (1.46 mmol/L). In the 4 days following the start of amphoB the patient developed vomiting, severe malaise and muscle aches. A progressive decrease in HCO_3 and an increase in anion was noticed. At day 5, patient developed hypotension, severe dyspnea and stupor. LA was diagnosed (lactate 13.0 mmol/L, HCO_3 5.1 mmol/L, pH 7.22). HAART and amphoB were stopped and vitamins, fluids, HCO_3 and dialysis were started. At day 12, lactate (0.9 mmol/L), pH (7.44) and HCO_3 (24 mmol/L) were normalized and amphoB (but not HAART) was restarted. During the following 3 days, lactate increased again (2.6, 4.3 and 6.0 mmol/L). Despite maximal therapy, lactic acidosis persisted. The patient died 5 weeks after start of amphoB due to LA and multiorgan failure.
Discussion: AmphoB causes distal renal tubular acidosis type I, with a decreased renal H+ excretion. No case of LA is linked to amphoB in the literature. In this patient, the start of amphoB coincided with the abrupt onset of LA. It may be prudent to monitor patients under HAART and amphoB closely for LA.

Viral vaccines

P903 Decay of measles maternal antibody in infancy in Indian children

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Objective: Measles continue to be an important cause of mortality and morbidity in developing countries including India in spite of the availability of an effective vaccine. The effectiveness of the vaccination against measles greatly depends on the level of measles maternal antibody (MMA) in the blood during infancy. This study was done to determine the Geometric Mean Titer (GMT) of MMA and its decay pattern in infancy in Indian children.
Methods: Cord blood samples from 200 new born infants and blood samples collected from 122 children below 1.5 years who neither had natural measles nor received measles vaccination was collected. Measles neutralizing antibody in blood was detected by a microneutralization assay using Edmonston-Zegreb vaccine strain of Measles virus (used in India) and the results were expressed as milli International Units/Ml (mIU/mL) with reference to the WHO 2nd International standard 1990 Anti-measles antibody.
Results: The anti measles neutralizing antibody titer varied from 62.5 to 16,000 mIU/mL. The GMT of maternal measles antibody titer in the cord blood was 942.80 ± 3.364 mIU/mL GMT of maternal measles antibody titer in the infants blood showed a statistically significant reduction with an increase in age during the early part of the infancy and touched the lowest 79.27 ± 1.756 mIU/mL by 7th month and there after remained in the vicinity of 125 mIU/mL (Fig. 1). At all age groups and in cord blood there was a proportion of samples showed a GMT less than 125 mIU/mL and the number of children who had low GMT was directly proportional to increase in age. On the contrary, few children had high GMT even after 9 months of age.

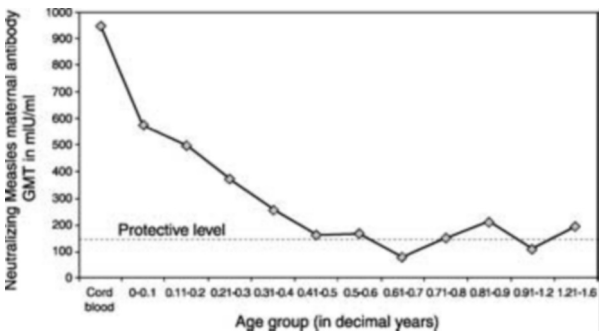

Figure 1 Decay of Neutralizing Measles Maternal antibody in infancy.

Conclusion: This study supports the earlier findings that GMT of measles maternal antibody in infants blood decay as age advances and reaches a low level below 125 mIU/mL by the end of 6th month. We found that few children had below 125 mIU/mL even before they reach 6 months of age while some children possess a very high level of measles antibody in their blood even after 9 months of age. These findings explain the occurrence of measles in infants below 6 months as well as vaccine failure in children vaccinated at 9 months. Hence, determination of absolute titers of Measles neutralizing antibody in infants blood prior to vaccination may prove to be helpful in deciding the age of vaccination to get the maximum seroconversion. This strategy could go a long way in achieving eradication of measles from the world.

P904 Prevalence of measles maternal antibody in newborns in Udupi, South India

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Objective: Measles is the major cause of vaccine preventable deaths in infants in the world. The ability of maternal antibody to measles virus to interfere with seroconversion after vaccination is well known. This study was conceived to determine the absolute level of measles maternal antibody (MMA) in the cord blood of newborns in a south Indian district Udupi and to find out the factors affecting the MMA level.
Materials and method: Cord blood samples from 1031 new born infants was collected. Measles neutralizing antibody in cord blood was detected by a microneutralization assay using Edmonston–Zegreb vaccine strain of Measles virus (used in India) and the results were expressed as milli International Units/mL (mIU/mL) with reference to the WHO 2nd International standard 1990 Anti-measles antibody. The association of maternal and infant related factors with MMA level in cord blood was compared using ANOVA by a statistical program SPSS.
Results: The antimeasles virus antibody titer in cord blood ranged from 62.5 to 16,000 mIU/mL with a geometric mean titer (GMT) of 1223 ± 3.467 mIU/mL. While 99% of cord blood samples showed a titer 125 mIU/mL or more, 1% of samples had a titer of 62.5 mIU/mL or less. Various maternal and infant related factors such as mother's age, parity, mode of delivery, mother's HIV status, mother's blood group, baby's sex, birth weight and gestational age did not show any statistically significant association with MMA titers in cord blood.
Conclusion: The GMT of MMA titer in cord blood was 1223 ± 3.467 mIU/mL. Considering the titer 125 mIU/mL as the minimum detectable level the prevalence of MMA in cord blood of infants was 99%. It is interesting to note that 1% of cord blood had a titer less than 125 mIU/mL indicating their susceptibility to natural measles well before the age of routine vaccination. On the other hand, many samples recorded a very high level of MMA. We did not

find any of the maternal or infant related factors significantly influencing the level of MMA in cord blood. Determination of absolute MMA in cord blood may a useful predictor in deciding the schedule for measles immunization to get maximum benefit in terms of seroconversion and coverage.

P905 Seroprevalence against measles, mumps and rubella in vaccinated children with a dose of MMR according to the type of vaccine received

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Objective: To evaluate the seroprevalence and immunogenicity against measles, mumps and rubella in children vaccinated with one dose of MMR according to the type of vaccine received.
Methods: 122 children aged between 2 and 5 years and with one confirmed dose of MMR vaccine were studied. The detection of IgG against measles, mumps and rubella was carried out by indirect ELISA (Enzygnost, Dade Behring GmbH, Germany). The children were grouped according to the type of vaccine administered: Triviraten ($n = 78$), Triple Vaccine MSD ($n = 41$) and Priorix ($n = 3$). The seroprevalence percentages and the geometric mean antibodies concentration (GMC) against each virus were calculated with confidence intervals of 95% (CI95%).
Results: The global seroprevalence against mumps, measles and rubella was, respectively, of 55.7%, 92.6% and 98.4%. In children vaccinated with the Rubini strain (Triviraten), the seroprevalence (43.6%, CI95% 32.6–55.3) and the GMC of antibodies against mumps (354, CI95% 295–426) were significantly lower ($P < 0.01$) regarding the children vaccinated with Jeryl Lynn (Triple MSD) (seroprevalence 78.1%, CI 62.0–88.9; and GMC 1000, CI95% 676–1479). No differences in the seroprevalence for measles or rubella were obtained. However, the GMC of antibodies against measles were significantly lower ($P < 0.01$) in children vaccinated with the Edmonston Zagreb strain (Triviraten) in relation to the children receiving the Enders strain (Triple MSD) (977; CI95% 758–1258 vs. 2089; CI95% 1412–3090). By the contrary, the GMC of antibodies against rubella were significantly lower in children vaccinated with Triple MSD (33; CI95% 25–42) than in the children vaccinated with Triviraten (89; CI95% 74–107) (both vaccines include the Wistar RA 27/3 strain).
Conclusions: The strain of mumps vaccine administered may have important implications in the real status of protection (less protection in children vaccinated with the Rubini strain). The meaning of the GMC differences for both measles and rubella viruses depending the vaccine administrated is more difficult to establish, but seems to be clinically minimum.

P906 Measles vaccination in Greece: seroprevalence in general population

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Objectives: Measles is primarily child's disease caused by an RNA virus and is the major cause of mortality in malnourished infants and of morbidity in immunosuppressed patients (HIV-patients) The aim of the present study was to evaluate the coverage rate of measles vaccine in order to obtain data that could be used to determine the vaccination policy in Greece.
Materials and methods: A total of 775 serum samples were screened for IgM and IgG antibodies which were obtained from 496 males and 279 females aged from 0 to 83 years. The IgM and IgG antibody titers against measles were measured by enzyme immunoassay method (ELISA) (EIA-PanBio). Positive were considered the samples having values of greater or equal to 11 PanBio units, whereas negative were considered the samples having values less than or equal to 9 PanBio units.
Results: Measles specific IgG antibodies were found to be positive in 81% (627/775) of the serum samples. Particularly, IgG antibodies were found in 82.5% (409/496) and 78.1% (218/279) in the male and the female groups, respectively. Low vaccination coverage, 58.3% (7/12) was observed in children 0–2 years of age, whereas, 80% (36/45) of the children 2–5 years of age were shown to be covered by the vaccine. Measles specific IgM and IgG antibodies were found in 35 serum samples (22.1%), obtained from children aged 0–20 years. Measles specific IgM antibodies were detected only in five serum samples (4.9%) from children 6–20 years of age, whereas, only two samples were found to have IgG and IgM antibodies in ages >20 years old. A total of 19% from the examined population were not immunologically covered (IgM and IgG negative) and belonged to all age groups examined showing a great susceptibility to the age group of 40–50 years (26%).
Conclusions: The total population immune response against measles virus was found to be 81%. The immune response was found not to be satisfactory especially for the age groups 0–2 years (58.3%) and 40–50 years (74%) according to the WHO recommendations that vaccination routine coverage of the population should be >90%.

P907 Serologic response to three doses of recombinant hepatitis B vaccine in 2–5-year-old-population in the Community of Madrid

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Objectives: To describe the serologic response to the recombinant hepatitis B vaccine in the 2–5 years old population living in the Community of Madrid (CM) and who were vaccinated with a three-dose regimen, starting at birth.
Methods: 174 children aged 2–5 who had received three doses of vaccine, starting at birth, were selected among those who participated in the latest Seroprevalence Survey carried out in the CM from September 1999 to April 2000. Antibodies to hepatitis B virus antigen (AntiHBs) were measured through EIA (Anti-HBs VITROS® ECI, Ortho Diagnostic Systems Inc.). Levels of antiHBs higher than 10 IU/mL were considered as protective. Persistence of levels in a 2–5 years interval from the last dose is analyzed.
Results: Prevalence of antiHBs was 86.2% (CI 95%; 80.2–91.0%) in the sample studied. No decreasing trend was found within the time interval considered.
Conclusions: The low prevalence detected could be due to the loss of antibodies after vaccination, as other authors have already observed, especially during the first year. However, serologic measurements were not made immediately after vaccination, so that the initial response cannot be known. Therefore, these results could be caused by an inadequate administration or conservation of vaccine.

P908 Persistence of anti-HB antibodies in healthy Iranian children vaccinated with recombinant hepatitis B vaccine and response to a booster dose

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Objectives: Long-term protection against hepatitis B virus (HBV)is dependent on persistence of anti-HBs antibodies and/or strong immunologic memory. The aim of this study was to evaluate the persistence of anti-HBs antibodies in healthy Iranian children 5 years after primary vaccination and the response to a booster dose using recombinant HB vaccine.
Methods: Originally from February to October 1995 triple 10 μg doses of a recombinant HB vaccine (Engerix-B) were administered i.m. at 0, 1.5 and 9 months of age to 81 healthy Iranian neonates. Blood samples were collected in November 2000, 5 years after completion of primary vaccination course. Immediately a single booster dose of the same concentration was administered i.m. to all children and peripheral blood was taken 4 weeks after booster vaccination again. The level of anti-HBs antibody was quantitated in serum by sandwich ELISA.
Results: At 5 years after completion of primary vaccination course, seroprotection (anti-HBs > 10 IU/L) was observed in 61/81 (81.5%) of children. After booster dose 100% vaccinees developed protective level of antibody and geometric mean titer (GMT) rose from 206 IU/L to 1278 IU/L. The postbooster GMT was found to be significantly higher in comparison with prebooster mean concentration ($P < 0.0000001$). Before and after booster vaccination both seroprotection rate and GMT were found to be higher in females compared with male children but differences were not statistically significant.
Conclusion: These results indicated the existence of an effective immunologic memory over period of 5 years after primary vaccination with HB vaccine.

P909 Antibody response of an adjuvanted influenza vaccine in COPD patients on steroid therapy

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Objectives: The currently used Influenza vaccine formulation is known to have limited immunogenity in high-risk groups as COPD patients. The efficacy is known to range between 60 and 70%. Therefore, a new vaccine formulation was developed combining subunit influenza antigens with a MF59 adjuvant emulsion (Fluad®, Chiron Vaccines, Germany). The aim of the study was to compare the antibody response after vaccination with the new adjuvanted influenza vaccine (AIV) in three different groups of patients.
Methods: Elderly patients (>60 years) with COPD were included in the following groups: (1) subjects without steroid therapy (control group, $n = 44$); (2) patients on inhalative steroids (group II; $n = 90$); (3) patients on systemic steroids ($\geq$10 mg prednisolone/day; group III; $n = 40$). All enrolled subjects were vaccinated with the AIV. The vaccine contained the components of the virus strains A/NewCaledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2), B/Sichuan/379/99. Antibody titers were determined at baseline, 4 weeks and 6 months after vaccination. Measurements were performed by hemagglutination inhibition assay. Nonresponders were defined as subjects with a less than twofold antibody titer increase and/or a titer level <40.
Results: Of $n = 174$ initially enrolled patients $n = 162$ (93.1%) completed the study (male $n = 88$; female $n = 86$; mean age 71 years). We found a significant ($P < 0.05$) increase of mean geometric antibody titers for all three strains in all groups 4 weeks after vaccination. After 6 months antibody titers against influenza A (H1N1, H2N3) fell close to baseline, only Influenza B (B/Sichuan/379/99) antigens induced a sustained immune response. Differences between patient groups were not significant. We found an important rate of nonresponders (after 4 weeks: 58% (H1N1); 70% (H3N2); 51% (B); after 6 months 92% (H1N1); 91% (H3N2); 48% (B)).
Conclusion: Antibody response to vaccination after 4 weeks was sufficient in all groups regardless a concomitant steroid therapy. Despite this we found an important rate of nonresponders after 4 weeks and 6 months.

P910 Rabies antibody response achieved by different rabies prophlaxis methods

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Introduction: Rabies is one of the most lethal infectious diseases known. Rabies immunoglobulin (RIG) and vaccine are the only agents that can be used for treatment or postexposure prophylaxis. Two problems, however, have continued to exist for rabies prophylaxis. RIG is very expensive and not readily available where it is needed.
Objective: We aimed to compare the rabies antibody titers achieved by two different postexposure prophylaxis program; RIG and five dose standard vaccination (on days 0, 3, 7, 14, 28) versus 2-1-1 schedule (on days 0, 7, 21).
Methods: Patients with a history of animal bite admitted to outpatient clinic of Health Minister Ankara Training and Research Hospital, Department of Infectious Disease and Clinical Microbiology, between September–December 2001, were included in the study. Patients with underlying disease and medication history were excluded. Postexposure prophlaxis was given according to Rabies Prophylaxis Guideline of Turkish Health Ministry which was based on WHO and CDC recommendations. Patients with class III exposure were treated with RIG and five dose standard vaccination. 2-1-1 vaccination schedule was applied to patients either that RIG could not be used or admission from low endemic area for rabies. All candidates were vaccinated with either human diploid cell vaccine or Vero-cell culture vaccines. Blood samples were collected on days 7 and 28 after vaccination for each patient. Serum rabies antibodies were detected by ELISA, Platelia Rabies (Diagnostics Pasteur). The cutoff value was 0.5 IU/mL.
Results: A total number 83 patients were divided into two groups; There were 43 and 40 patients in group I and group II, respectively. 2-1-1 vaccination regimen was applied to group I and RIG and five dose standard vaccination to the other. At day 7, seroconversion of rabies antibodies was detected in 23 of 43 (53%) patients in group I and 12 of 40 (30%) patients in group II. Although seroconversion rate in group I was 1.76 times greater than group II, this difference was not statistically significant ($P > 0.05$). At day 28, seroconversion rates were 95 and 90%, respectively ($P > 0.05$).
Conclusion: Postexposure rabies prophylaxis should begin as soon as possible. As seroconversion rates of both regimens are inadequate at early period of postexposure, prompt local treatment of all bite wounds and scratches might be lifesaving. In the absence of RIG, applying 2-1-1 dose vaccination schedule may be an alternative to the standard vaccination.

P911 Protection of mice against a lethal HSV-1 challenge by immunization with DNA vaccine

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Objective: Nucleic acid based vaccines represent a novel approach to control of infectious diseases. The immunogenecity and protective efficacy of a DNA vaccine encoding herpes simplex virus type 1 (HSV_1) glycoprotein D (gD-1) under the control of CMV promoter was studied.
Methods: BALB/c mice immunized three times by intramuscular injection with pcDNA3-gD that contains full length of HSV-1. Humoral immune response was detected in sera taken from immunized mice.
Results: Following each dose of vaccination sera were analyzed with virus neutralization test. The sera were shown considerable antibody titer after second and third dose of vaccination. Immunized mice were 85% protected against lethal challenge with a virulent HSV-1 isolated from an Iranian patient.
Conclusion: DNA vaccination does induce a protective immunity and Ab response against HSV-1, which could be maintained by expression of gD gene in muscle cells.

P912 Nucleic acid vaccine encoding glycoprotein B of HSV-1 can protect mice from HSV-1 challenge

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Objective: One of the most important immunogenic proteins of HSV-1 is glycoprotein B (gB), so it is being used in recombinant vaccines to induce protectivity against virus. We constructed an expression vector containing gB-1 and used it as a DNA vaccine.
Methods: A clone carrying full length of gB-1 was digested with BamH1 to separate gB-1 gene. The gene was inserted in pcDNA3 and its ability in expression of gB was tested by immunofluorescence in COS-7 transfected cells. BALB/c mice were immunized with 90 microgram of purified DNA for three times. Humoral response of the immunized mice were measured with virus neutralization test.
Results: One hundred percent of serum samples contained antibody titer and 100% of immunized mice were protected against lethal dose of HSV-1 challenge, although 25% of them showed symptoms of infectivity.
Conclusion: The results demonstrated that gB DNA vaccine is highly efficient in production of antibody and suggests that it may have potential as a protective recombinant vaccine.

Non-molecular diagnostics: respiratory, blood and staphylococcal

P913 Evaluation of *Burkholderia cepacia* selective agar (BCSA) for the isolation of *Burkholderia cepacia* from sputum in pediatric patients with cystic fibrosis

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Objectives: Rapid isolation and identification of bacteria belonging to the *Burkholderia cepacia* complex is important for appropriate management of cystic fibrosis (CF) patients. Since *B. cepacia* isolates grow quite slowly on conventional medium such as MacConkey, and are often mixed with rapid growing microorganisms such as *Pseudomonas* spp., the use of a selective medium is recommended to increase the recovery rate of these bacteria from respiratory samples. The purpose of this study was to evaluate the performance of two selective media, a recently designed medium BCSA (bioMerieux, France) and a commercially available medium, PC agar (AES Laboratoire, France) in comparison with a conventional MacConkey agar (Oxoid, France).
Methods: A total of 175 specimens of respiratory secretions from 135 pediatric CF patients were evaluated prospectively. Aliquots of 10 µL of sputum were streaked in parallel on BCSA, PC agar and MacConkey. Bacterial strains were identified at species level by conventional biochemical methods, the *B. cepacia* complex status was assessed by PCR-RFLP of the 16S rDNA.
Results: *B. cepacia* was isolated from 7 of 175 (4%) samples examined, representing 3 of 135 patients with CF (2.2%). The seven *B. cepacia* strains were detected on BCSA while six were detected on PC and MacConkey agar. On MacConkey five of the six strains were in mixed-culture with pseudomonads while on BCSA and PC agar, all the strains were in pure culture. A total of 11 (8.1%), 39 (28.8%) and 163 (100%) of other microorganisms grew on BCSA, PC and MacConkey agar, respectively. BCSA and PC agar showed the same global performance of selectivity concerning Enterobacteriaceae and pseudomonads. BCSA showed a better selectivity than PC agar concerning, mainly, cocci (12 vs. 1), molds (14 vs. 3) and yeasts (4 vs. 0). The low rate (2.2%) of colonization with *Burkholderia cepacia* complex, found in this study, confirms previous findings concerning pediatric CF patients.
Conclusion: The three media were, at the time of the present study, quite similar in term of sensitivity (because of the low rate of colonization in our pediatric patient). We are currently extending our population studied to adult CF patients from other centres in an attempt to further discriminate the sensitivity rate of the three media. However, concerning the specificity, our present data revealed that BCSA offers a real advantage toward the other media in term of easiness for the detection of *Burkholderia*.

P914 Evaluation of a new GVPC medium for the detection of *Legionella* in clinical and environmental samples

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Background: Community acquired or epidemic legionellosis often leads to severe pneumopathy with a possible mortality rate of 30% in case of nosocomial infections. The isolation of the strains from clinical samples allows the diagnostic and also the determination of the origin of the contamination by comparing the clinical and environmental strains with epidemiologic markers. The *Legionella* culture is therefore essential in clinical and environmental contexts. The GVPC medium from bioMerieux (LB) was evaluated in comparison with the GVPC medium from Oxoid (LO) routinely used in our laboratory.
Methods: The detection of *Legionella* was performed on 100 clinical samples (CS) and 104 water samples (WS). The detection of the *Legionella* in CS was performed using culture in our laboratory according to the GBEA protocol (French recommendations, MO LE 019). The waters were analyzed according to the technique described in the ISO 11-731 norm. The two media have been tested in parallel for all the samples allowing a comparison of specificity and selectivity.
Results: Among the 100 analyzed CS, 3 *Legionella* positive cultures were found on both media. The number of colonies was more important on the LB medium, with less contaminants than the LO medium: 50/60. Among the 104 tested WS, 55 were positive for *Legionella pneumophila* for the LB medium and 52 for the LO medium. At day 10 (end of the analysis) the *Legionella* enumeration was equivalent. However, *L. anisa* was better detected on the LB medium 16/8 with a more important colony number 12/2. Contaminants such as Gram-negative bacilli, Gram-positive bacilli and cocci were in smaller numbers on the LB medium: 12/14. Less moulds were found on the LO medium: 41/8.
Conclusion: For the WS, the LB medium showed better performance than the LO medium except for mold contamination at 10 days. For the CS, the LB medium was more selective, with less contaminants. The culture of *Legionella* from clinical samples is difficult. It is therefore important to be able to detect the *Legionella* colonies as soon as possible with few contaminants since they may mask the specific colonies of *Legionella* growing in 3 days. Moreover, as *L. anisa* is responsible for clinical cases, it is helpful to be able to detect this bacteria on the LB medium.

P915 Rapid identification of *Legionella pneumophila* serogroups by latex agglutination

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The sanitary hot water networks are often colonized up to 60% by *Legionella* belonging to all species. Among the isolated strains, a large number of *Legionella pneumophila* (Lp) remains unidentified due to cross-reactions with the direct fluorescent assay (DFA) which are the identification techniques used. Moreover, there is a lack of commercialized reagents for the identification of *Legionella*. We have developed a new reagent for a fast identification of Lp strains as well as clinical and environmental ones. At the French Reference Center, about 150 *Legionella* clinical strains have been identified each year (95% of *Legionella pneumophila* strains) and more than 3000 environmental strains of which 66% are Lp. We developed the following latex reagents: 1 monovalent Lp serogroup 1, 1 polyvalent Lp 2-14 and a monovalent reagent for each serogroup from Lp 2 to Lp 15. The evaluation of these reagents has been done using 16 reference strains and 367 other strains (27 clinical and 340 environmental strains). Using the latex reagents, 19 *Legionella nonpneumophila* strains produced no agglutination with Lp 1 and Lp 2-14 reagents. Three hundred and forty-eight Lp strains have been identified (78 Lp 1, 71 Lp 6, 65 Lp 3 and 64 Lp 8) as well as 4 Lp 2, 7 Lp 4, 10 Lp 5, 1 Lp 7, 5 Lp 9, 2 Lp 10, 4 Lp 12, 2 Lp 14 and 4 Lp 15, which were never found before (Table). In conclusion, the different latex reagents used have allowed us to identify 95% of Lp strains. The agglutination latex test takes 5 min while the DFA techniques requires 3 h. Among the strains isolated from water, the most frequent serogroups are Lp 1, Lp 6, Lp 3 and Lp 8, that corresponds to the clinical chart.

Table 1 Comparative study of the identification by DFA and latex reagent

Identification	DFA (%)	Latex (%)
Lp ser 1	92	98
Lp 2–15	9	94
Lp 1–15	27	95

P916 Comparison of indirect fluorescent antibody assay and culture method

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Objectives: Pulmonary nocardiosis is an acute or suppurative chronic disease, caused by a soil-borne aerobic actinomycete called Nocardia. The bacterium is an opportunistic pathogen in immunocompromised hosts. The present investigation was planned to detect nocardiosis in immunocompromised patients who had been confined in Shariati Training Hospital (Tehran), using indirect immunofluorescence assay (IFA) and bacterial culture methods. Comparison of the two methods and correlation between the antibody titer with the statistical and epidemiological data was also investigated.

Methods: One hundred and one patients with advanced symptomatic pulmonary infection were studied during a period of 10 months. All the patients were tested for their sputum, bronchoalveolar lavage (BAL) and blood sera. From each sample three thin smears were prepared for microscopic observations. The samples were cultured in Sabouraud dextrose, blood and paraffin agar. The detection of antibody against Nocardia asteroides was performed in all study groups, using IFA assay. Medical histories of patients were also recorded in questionnaires for further data analysis.
Results: Nocardia asteroides was isolated from only one patient suffering from Wegner vasculitis with an antibody titer of 1/512 in serum. Among the 41 patients suspected for nocardiosis with an antibody titer of at least 1/64 in sera detected by IFA, there were 26 men (63.4%) and 15 women (14.8%). The age of the patients varied from 7 to 80 years. Those who had reasonable antibody titers included 15 housewives (36.5%) and 9 workers (21.9%). Furthermore, in vitro investigation for differentiation of the isolate was performed and confirmed that the organism that grew on primary media was Nocardia asteroids complex.
Conclusion: Our results revealed that the bronchopulmonary infections which occur in high risk patients (T-cell deficiencies, long-term corticosteroid therapy, immunocompromised hosts, HIV infection, organ transplantation, etc.), was an important index for primary diagnosis of nocardiosis. As the important finding of the research, antibody at a titer of 1/64 could be proposed as a criterion for the diagnosis of the infection. The probability of nocardiosis was proposed, when antibody titer was less or more than 1/64.

P917 An indirect fluorescent antibody assay against *Nocardia* complex

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Tehran, IR

Objective: Nocardiosis is an acute or suppurative chronic disease caused by a soil-borne actinomycete called *Nocardia*. Species of *Nocardia* are aerobic, Gram-positive, partially acid fast bacilli. *Nocardia asteroides* which is the dangerous and most frequently pathogen, infects humans through the respiratory tract. The bacterium is primarily an opportunistic pathogen that causes the infection in patients with underlying immunodeficiencies.
Methods: The present investigation is a cross-sectional study conducted on a population consisted of 300 subjects including 200 hospitalized individuals' patients, nurses and healthcare workers from Imam Khomeini hospital, and 100 health adult blood donors. None of the patients had already been diagnosed to be affected by *Nocardia*. The main purpose of the study was to detect antibody against *Nocardia* in all study groups, using indirect immunofluorescent assay (IFA). Correlation between the antibody titer against *Nocardia* with age, sex, occupation, chronic pulmonary infection, and corticosteroid therapy was also investigated.
Results: Our results demonstrated four patients suffering from different infections, including TB, mycetoma, chronic pulmonary and chronic obstructive pulmonary diseases were IFA positive. None of the high risk hospital personnel who were working in close proximity to the areas infected with *Nocardia*, were found to be IFA positive. Meanwhile there was no positive result in a group of patients ($n = 34$) who were under corticosteroid therapy.
Conclusion: Finally, considering the small sample size of the IFA positive cases no significant association between the IFA results and age, sex, occupation and clinical conditions of the subjects could be established.

P918 Rapid antigen assay for the diagnosis of severe pneumococcal disease in patients from a university hospital

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Study objective: To evaluate the usefulness of a *Streptococcus pneumoniae* antigen urinary detection assay for the diagnosis of severe pneumococcal disease.
Methods: We retrospectively reviewed the records from the Microbiology laboratory for patients who had pneumococcal antigen assay (Binax NOW, USA) performed between January 1, 2000, and October 22, 2002. We selected for analysis those patients who had also blood cultures taken during an interval of 10 days before or after urinary antigen was performed. We reviewed clinical charts from patients with positive blood cultures, positive urine test, or both, to determine the clinical syndrome of the patients.
Results: Six hundred and nineteen patients had a urinary antigen test performed during the study period. Three hundred and thirty patients had also blood cultures taken and were evaluated in further analyzes. The assay gave a positive result in 14 of 16 patients with pneumococcal bacteremia, yielding a sensitivity of 87.5%. The test result was negative in 276 of 314 patients with negative blood cultures (specificity 87.9%). Patients with a positive test and negative blood cultures had pneumonia (35 cases), and upper respiratory tract infection (3 cases). Both patients with a positive blood culture for *S. pneumoniae* but antigen test negative had pneumonia. Patients with both tests positive had pneumonia (12 cases) and pneumococcal meningitis (2 cases, both with positive CSF cultures for *S. pneumoniae*).
Conclusion: The *S. pneumoniae* antigen detection assay is useful for the diagnosis of severe pneumococcal disease in the defined population.

P919 Isolation and identification of *Moraxella* (*Branhamella*) *catarrhalis* in a routine laboratory from expectorated sputum samples within 48 h

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Ibb, YE

Objectives: To set economic, practical, rapid and nonlaborious laboratory procedures for the isolation and identification of *M.* (*B.*) *catarrhalis* from sputum and other samples.
Methods: Using selective cellular criteria and a Gram-stain directed culture method for selection of noncontaminated and representative sputum samples. Predominant Gram-negative diplococci on microscopy and predominant growth of typical colonies on 5% human blood agar after 24 h of aerobic incubation at 37 °C, initially noted. Confirmation achieved by positive rapid oxidase test (rubbing colonies on oxidase impregnated filter paper pieces), and by positive DNase test (growth carrying needle stabbed into microtiter wells containing toluidine *O*-DNase agar) within less than 24 h of incubation.
Results: Using the above method, 26 clinically significant isolates of *M.* (*B.*) *catarrhalis* were isolated and identified from 300 adults with productive cough, within 48 h of sputum samples receipt. The laboratory results were constant and the tests were reliable for all the isolates. No need to do carbohydrate fermentation tests or to test growth on nutrient agar at room temperature or on modified Thayer–Martin medium.
Conclusions: We recommend using the selective policy for sputum samples acceptance, Gram-stain directed culture method for proper isolation, and economically prepared rapid oxidase and DNase tests for final identification. This is most important in developing countries.

P920 New cytological criteria for the microbiological diagnosis of acute exacerbation of chronic bronchitis

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Objectives: Re-evaluation of the validity of macroscopic and microscopic examination of sputum specimens from patients with acute exacerbation of chronic bronchitis (AECB) and development of new cytologic criteria for quality assessment of specimens.
Methods: Two hundred and fifty-two sputa from patients with AECB were inspected macroscopically (purulent, slightly purulent, mucoid) and examined by microscopy (cytology, Gram) and quantitative culture. Two methods for interpretation of specimen adequacy were applied: (i) Bartlett criteria (<25 squamous epthelial cells (SC) and >25 neutrophils (PMN) per low-power field) and (ii) Heckeshorn criteria developed by our group which rely on the ratio between SC and PMN: a sputum is considered of high quality, when it shows <20% SC and simultaneously >50% PMN per field.
Results: As expected, positive culture was most frequently associated with purulent exacerbations (47 of 70, 67%). However, slightly purulent and mucoid specimens revealed an unexpected high isolation rate of pathogens, 52 and 25%, respectively. With respect to the cytological Bartlett criteria 76% of purulent (53 of 70), 42% of slightly purulent (18 of 42) and still 28% of mucoid (39 of 140) sputa could be considered as acceptable specimens. Using the new criteria, we were able to classify an additional 26% of sputa as acceptable. Of 110 acceptable specimens according to the Bartlett criteria 65 yielded growth of bacterial pathogens in high concentrations (>105 cfu/mL). This figure could be increased by 20% by using the new method which involves ratios of

SC and PMN. Microscopy of Gram stains was highly reliable in diagnosing either *H. influenzae* (microscopy 22, culture 20) or *M. catarrhalis* (microscopy 14, culture 14). The Heckeshorn criteria were clearly superior to the Bartlett criteria in predicting significant cultural results (84 vs. 65).
Conclusions: Purulent sputum can be considered to be the specimen with highest adequacy for the diagnosis of AECB, but nevertheless nearly a quarter of nonpurulent sputa could be evaluated as well. The new cytological criteria based on ratios and relative cell counts in percentage increased the number of potential pathogens which could be interpreted as causative agents of the acute exacerbation by an additional 30%. Since the Bartlett criteria failed to identify a considerable portion of patients which could otherwise be treated specifically, we conclude that the Heckeshorn criteria are superior to the Bartlett criteria and will improve the diagnosis of AECB.

P921 Rate of false negative blood cultures in BACTEC 9000

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Objective: In 2000 Klaerner et al. (1) from Munich reported that BacT/Alert failed to detect nonfermentative Gram-negative bacteria. We performed a two center prospective study to evaluate the rate of false-negative blood cultures (not detected by BACTEC but positive on terminal subculture) in two routine laboratories.
Material and methods: One hospital based laboratory and one laboratory servicing a number of hospitals planned to prospectively study 1000 pairs of blood cultures each. Among other data, information on time of inoculation, entry into system, antimicrobial treatment and time to detection by BACTEC 9000 were recorded and analyzed.
Results: (see table below):

Parameter	Center 1	Center 2	Total
Patients (*n*)	467	876	1343
Cultures (*n*)	990	889	1879
Bottles (*n*)	1984	1923	3907
Average transport time (h)			
All	21.5	21.3	21.4
True positives	19.3	21.6	20.7
False positives	2.0	30.2	21.0
True negatives	21.2	21.1	21.2
False negatives	71.8	30.9	55.1
Time to detection (h)	27.7	16.3	20.7
True positives (%)	9.1	14.1	11.6
False positives (%)	0.1	0.2	0.2
True negatives (%)	90.0	85.1	87.6
False negatives (%)	0.8	0.6	0.7

27 bottles were classified as false negative, 7 of these had a positive cohort bottle [*S. aureus* (N = anaerobic), *E. cloacae* (A = aerobic), *E. faecalis* (N), *C. albicans* (N), *B. cepacia* (N)] and two strains of *P. aeruginosa* (N), 4 of these 7 were not expected to grow in these media. One *C. glabrata* (N) without a positive cohort bottle was also not expected to grow in this media. 15/27 were from patients on antimicrobial therapy. Four were contaminants. Fifteen of the false negative bottles had a transport time of >48 h. Only one true significant false negative (polymicrobial: *H. alvei*, *E. faecium*, *C. perfringens*) occurred in the study.
Summary: The rate of clinically relevant false negative blood cultures (pathogen not detected in cohort bottle) in bottles that were entered into BACTEC 9000 within 48 h after inoculation is 0.03%. All efforts need to be directed to prompt transportation of material to the microbiology laboratory.

Reference

1. H.-G. Klaerner, U. Eschenbach, K. Kamereck, N. Lehn, H. Wagner, T. Miethke. Failure of an automated blood culture system to detect nonfermentative Gram-negative bacteria. *J Clin Microbiol* 2000; 38 (3): 1036–41.

P922 A comparison of commercial DNA extraction kits for the extraction of bacterial genomic DNA from whole blood samples

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Objectives: A rapid diagnosis of the causative organism is crucial to the treatment and recovery of individuals suffering from bacterial septicemia or meningitis. Advances in molecular biology have promoted the routine use of techniques such as PCR and DNA sequencing for the improved diagnosis and surveillance of microbial disease. Bacterial DNA can be extracted from the body fluids of patients with suspected bacterial disease and then amplified by PCR and sequenced to enable detection and identification of the bacteria responsible. The success of these reactions depends upon an efficient method of DNA extraction that produces pure, high quality DNA. Automation of the extraction process would also allow a more rapid throughput of clinical samples. In this study, five commercially available kits were compared for the extraction of bacterial genomic DNA from whole blood samples.
Methods: The five kits operate by 96-well binding plate, 96-well filter plate or metallic bead formats. Tests were carried out to determine the sensitivity and specificity of each kit. All kits were automated on the MWG Biotech Roboseq 4200PE liquid handling robot, and the ease of use and automation was compared. The PicoGreen assay was used to quantify the level of DNA in each extracted sample.
Results: All kits were used successfully and were fully automated. Overall efficiency was determined by the sensitivity, specificity, DNA yield, and throughput time for sample processing for each kit. The Bilatest DNA 2 kit and the Promega Wizard SV96 kit were most sensitive with the ability to detect down to 1–2 genome copy units per 100 mL sample. These kits also produced the highest yield of DNA and were the easiest to automate.
Conclusion: The Promega Wizard SV96 binding plate system and the Bilatest DNA2 metallic bead kit are both highly sensitive and produced good quality bacterial DNA at a desirable concentration for use in PCR and sequencing reactions. Furthermore, both kits are easily automated, and simple to use. They are capable of processing 96 samples in a short time scale without the need for manual intervention. This is highly beneficial to the patient as it allows labor to be concentrated in other areas and leads to a more rapid diagnosis of disease.

P923 Controlled clinical comparison of BacT/ALERT pediatric PF vs. adult FA bottles for culturing blood from pediatric patients

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Objectives: The pediatric BacT/ALERT PF medium (PF) (bioMerieux, Durham, NC, USA) is a nonvented aerobic culture medium designed to detect bacteria from the blood of pediatric patients. To determine whether a pediatric medium is necessary, we compared the performance of PF to the adult BacT/ALERT aerobic FA medium (FA) for the recovery of microorganisms as well as the time to detection of growth in samples of blood obtained for culture from children.
Methods: Activated charcoal is present in both PF (8.5%, w/v) and FA (6.5%, w/v) media. All bottles were weighed before inoculation and upon receipt; only bottles filled with similar volumes of blood were compared.
Results: Of 5221 bottle pairs received, 3667 (70.2%) contained comparable volumes of blood. Of 203 clinically significant (based on previously published criteria) isolates, 142 were detected in both bottles, 32 only in PF, and 29 only in FA (P = NS). No organism group was detected significantly more frequently in one medium vs. the other. Of 140 sets that were positive in both bottles within 3 days, the mean time to detection of pathogens from pediatric patients was 20.5 h in PF and 19.5 h in FA bottles. False-positive instrument signals were detected in 5 of 3667 (0.1%) PF bottles and 6 of 3667 (0.2%) FA bottles.

Conclusions: We conclude that BacT/ALERT PF and FA bottles are comparable for recovery of microorganisms from pediatric patients and that any potential advantage of the pediatric formulation was not detected in this study.

P924 Controlled comparison of two BacT/ALERT aerobic FA bottles vs. the combination of an aerobic and anaerobic FA bottle for the culture of equal volumes of blood from adults

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Objectives: Optimal distribution of a given volume of blood between media and different atmospheres of incubation for detection of bloodstream infections remains uncertain. To assess the overall yield and yield by microorganism group, we compared 20 mL of blood inoculated into two aerobic FA bottles (8–12 mL each) (FA/FA) vs. the same volume split between one aerobic FA bottle and one anaerobic FA bottle (FA/FN) (bioMerieux Inc., Durham, NC, USA).
Methods: At the bedside, 30-mL samples of blood from patients with suspected sepsis were inoculated equally into two FA bottles and one FN bottle. Only culture sets that had all three bottles filled with 8–12 mL of blood each were included in the study.
Results: Overall yield from 3841 three-bottle blood culture sets that included 360 positive cultures of clinical importance favored the FA/FN combination. *Staphylococcus aureus* ($P < 0.005$) and anaerobic Gram-positive and Gram-negative bacilli ($P < 0.025$) were detected more frequently in FA/FN, but yeasts ($P < 0.005$) were detected more frequently in FA/FA. For 171 pairs of FA and FN bottles in which both bottles were positive, the mean time to detection within 72 h was 18.3 h in FA and 18.0 h in FN. Of 151 contaminant isolates detected, there was no difference in contamination rates between the two systems. False-positive instrument signals were detected in 10, 9, and 11 (0.3%) of 3841 FA, FA, and FN bottles, respectively.
Conclusion: We conclude that the BacT/ALERT FA/FN bottle combination with 20-mL samples of blood yields better results than FA/FA for the detection of bacterial bloodstream infection in adults.

P925 Determination of serum procalcytonine and presence of bacteria in blood cultures in patients in a tertiary care hospital, Gdansk, Poland in 2002

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Objectives: To analyze serum procalcytonine (PCT) level and blood cultures in febrile patients hospitalized in Tertiary Care Hospital in Gdansk in 2002.
Methods: In the year 2002 we examined PCT level in 77 patients (pts). All of them were febrile (above 380C). We analyzed serum PCT level and blood cultures taken on the same day. We performed PCT analysis using the test PCT-Q (Brahms Diagnostica GMBH). Blood cultures were incubated in BacT/Alert system (bioMerieux). Microorganisms were identified by standard methods and disc-diffusion susceptibility testing was done according to NCCLS.
Results: We divided pts into four groups depending on their PCT level. In the first group (PCT level ≥ 10 ng/mL) 58.3% blood samples were positive, we isolated mostly *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) from blood cultures. In the second group (PCT level ≥ 2 ng/mL) 53.8% blood samples were positive, we isolated most often *Escherichia coli*, PA and *Streptococcus pyogenes*. In the third group (PCT level ≥ 0.5 ng/mL) 23.1% blood samples were positive, we isolated mostly PA and *Enterococcus* sp. In the fourth group (PCT level < 0.5 ng/mL) only 17.9% blood samples were positive, we often isolated *Enterococcus* sp. and *S. epidermidis*.
Conclusions: We can conclude that the correlation between serum PCT level and isolation of pathogens from blood cultures exists. We could not confirm correlation between serum PCT level and prevalence of Gram-positive or Gram-negative bacteremia.

P926 Biofilm production by *Staphylococcus epidermidis* at 37 and 32 °C

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Objectives: *Staphylococcus epidermidis*, the most prominent causative agent of catheter related bloodstream infections, enters the bloodstream from the skin insertion site or through the hub of catheter device. The ability of the organism to produce biofilm at 37 °C, which corresponds to the inner temperature of human body, has been well established. However, the superficial temperature of human organism is lower and is roughly averaged at 32 °C. The present study compared biofilm-producing capacity of *S. epidermidis* and biofilm formation by other staphylococcal species after incubation at 37 and 32 °C.
Methods: Quantification of biofilm formation by eight *S. epidermidis*, eight *S. aureus*, eight *S. sciuri* and six *S. haemolyticus* strains was performed by the modified microtiter-plate test. After overnight incubation in brain heart infusion broth poured in 96-well flat-bottomed plastic microplates, bacterial film was fixed with methanol and stained with crystal violet. The bound dye was released with 33% glacial acetic acid, and optical density was measured at 570 nm by using an automated microtiter-plate reader.
Results: Among *S. epidermidis* strains tested, six (75%) produced more biofilm when incubated at 32 than at 37 °C. On the other hand, only two (35%) *S. aureus* strains and one (16.7%) *S. haemolyticus* strain produced more biofilm when incubated at 32 than at 37 °C. All *S. sciuri* strains tested produced more biofilm when incubated at 37 °C.
Conclusion: In contrast to other staphylococcal species tested, *S. epidermidis* showed more abundant production of biofilm after incubation at lower temperature, which corresponds to the superficial temperature of human organism, than after incubation at 37 °C. The obtained results indicate that the capacity of *S. epidermidis* to produce biofilm at the temperature lower than 37 °C, should also be considered as a factor contributing to its marked ability to cause catheter related bloodstream infections.

P927 Optimalization of conditions for biofilm growth of *Staphylococcus epidermidis*, ica-operon positive and negative, on polystyrene surface

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Objectives: The aims of the study were to evaluate the applicability of different surface treatments for biofilm formation on hardened polystyrene material in different culture media and to evaluate the applicability for assessment of minimum biofilm eradication concentration of antibiotics (MBEC) in routine laboratory conditions.
Methods: The *S. epidermidis* strains isolated from hemocultures were used in this study. Isolates were grouped on the basis of presence of ica-operon, determined by PCR reaction, into two groups, ica-operon positive (20) and negative (20). All isolates were examined phenotypically by two methods – by Christensen's method and by the typical growth on agar with Congo Red. For the growth evaluation, the plates of polystyrene with 96 pegs that fit into standard microtiter-plates were used. The pegged plates were assigned for the microtiter assay of MBEC. The growth of biofilm was observed in six different variants – on unadapted pegs, on pegs adapted with abrasion by means of acetone and on pegs adapted with sulfonation by means of sulfuric acid, all in Tryptic Soya Broth and Brain Heart Infusion, respectively. The General Linear Models and Dunnett analysis were used for the data evaluation. All tests were examined at the 95% probability level.
Results: From 20 ica-positive strains 14 (70%) were positive with CRA method, 2 (10%) and 12 (60%) with Christensen's method, 0 and 10 (50%) with unadapted pegs, 0 and 12 (60%) with pegs adapted by abrasion, 3 (15%) and 14 (70%) with sulfonated pegs, all in TSB and BHI, respectively. From 20 ica-negative strains 17 (85%) were negative with CRA method, 18 (90%) and 20 (100%) Christensen's method, 20 (100%) and 15 (75%) with unadapted pegs 19 (95%) and 17 (85%) with pegs adapted by abrasion, 19 (95%) and 16 (70%) with sulfonated pegs, all in TSB and BHI, respectively.
Conclusions: Stronger biofilm formation was observable on pegs with adapted surface, where the sulfonation proved to be better than the chemical abrasion.

For the slime formation the nutrition-rich medium (BHI) proved to be more appropriate. Staphylococci form there a more vigorous slime layer. Despite the biofilm formation on the pegs, the layer was neither homogenous nor well adhered. For the routine laboratory use of the pegged plate it would be good to consider application of other materials.

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P928 Evaluation of *S. aureus* ID: a new chromogenic agar for the isolation and identification of *Staphylococcus aureus*

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Objectives: *S. aureus* ID medium (bioMerieux) is a new chromogenic agar designed for the enhanced isolation of staphylococci and the specific identification of *Staphylococcus aureus*. The medium contains selective agents for the inhibition of nonstaphylococci and a chromogenic substrate for alpha glucosidase produced by strains of *S. aureus* which are visualized as green colonies. The aim of this study was to evaluate the diagnostic utility of this medium with clinical samples for the isolation of *S. aureus*.

Methods: A total of 147 wound swabs were cultured onto blood agar (BA), aztreonam blood agar (ABA) and *S. aureus* ID medium (SAID) using a standardized inoculum. All culture plates were incubated for a total of 48 h at 37 °C and interpreted after both 18 and 48 h. The number of colonies of each organism type was recorded on each medium and all strains were identified by standard methods. Colonies of staphylococci were tested with latex reagent for the identification of *S. aureus*.

Results: After 18 h of incubation a total of 109 strains of *S. aureus* were isolated on any of the three media. Ninety-eight percent of these strains were isolated as green colonies on SAID medium compared with 75% isolated on BA and 92% on ABA. One further strain of *S. aureus* presented as cream colonies on SAID after 18 h of incubation and required 48 h of incubation for generation of pale green colonies. No further strains of *S. aureus* were isolated after extended incubation on BA or ABA. Only two non-*S. aureus* strains presented as green colonies and both strains were confirmed as *Micrococcus luteus*. After 18 h of incubation a total of 63 strains of coagulase-negative staphylococci were isolated on any of the three media. Ninety-seven percent of these strains were isolated on SAID medium compared with 51% on BA and 67% on ABA. SAID medium was more selective than ABA. No Gram-negative bacteria were recovered on SAID medium and most strains (94%) of enterococci were inhibited.

Conclusions: *S. aureus* ID medium was a useful diagnostic tool for the enhanced isolation of staphylococci from clinical samples. This was due to its high selectivity against enterococci and Gram-negative bacteria and its ability to differentiate *S. aureus* from coagulase-negative staphylococci based on colony color. It also allowed for the identification of *S. aureus* with high specificity.

P929 *S. aureus* ID: A new chromogenic medium for isolation of staphylococci and identification of *Staphylococcus aureus*

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Objectives: *S. aureus* ID (bioMerieux, Marcy l'Etoile, France) is a new, ready-to-use, chromogenic and selective medium designed for isolation of staphylococci and identification of *Staphylococcus aureus*. On *S. aureus* ID, identification of *Staphylococcus aureus* is based on the expression of alpha and beta-glucosidase, revealed by green-colored and pink-colored colonies. The detection of these two activities enables discrimination between *S. aureus* and other species. On this medium, *S. aureus* expresses alpha-glucosidase activity only, while other species express both activities, beta-glucosidase activity only or neither of them. Subsequently, *S. aureus* colonies are green whereas colonies of other *Staphylococcus* are purple/violet, pink or white. The aim of this study was to evaluate the performance of *S. aureus* ID medium in terms of fertility, selectivity, sensitivity and specificity in comparison with Mannitol Salt (MS, Chapman) medium.

Methods: *S. aureus* ID was compared with Mannitol Salt agar using a total of 350 bacterial and yeast strains, including clinical isolates but also strains from species rarely encountered in clinical samples or with particular phenotypes.

Results: The fertility of *S. aureus* ID was better than that of MS: colonies of *S. aureus* and coagulase-negative strains were larger and appeared earlier. In terms of selectivity, results for both media were similar with a slight advantage for *S. aureus* ID after 48 h of incubation at 37 °C (62.7% of inhibition vs. 53.6% for MS). The sensitivity of *Staphylococcus aureus* identification was higher on *S. aureus* ID medium with 90 and 100%, respectively, after 24 and 48 h, against only 12 and 84% with MS. There were more false positive results on Mannitol Salt; the specificity was in fact 77% after 48 h at 37 °C against 93.1% on *S. aureus* ID.

Conclusion: In conclusion, *S. aureus* ID medium enables the rapid and accurate identification of *S. aureus*, and this will help in early detection of MRSA strains.

Non-molecular diagnostics: gastro-intestinal infections

P930 The LOUIS test: a rapid biochemical based protocol for the screening of lactose nonfermenting colonies for the presence of *Salmonella* and *Shigella*

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Objectives: Identification of suspect *Salmonella* and *Shigella* colonies from primary isolation media is highly non-specific. The aim of this study was to develop a rapid screening test that would reduce the need for unnecessary biochemical identifications. This led to the formation of a protocol based on four rapid tests, lysine decarboxylase (LDC), o-nitrophenyl-beta-D-galactopyranoside (ONPG), urease production (URE), and indole (IND) (to be known as the LOUIS test).

Methods: Suspect oxidase negative colonies from DCLS and MacConkey agar primary isolation plates (Oxoid, England) were inoculated to 1 mL of sterile saline to give a suspension equivalent to at least McFarland no. 4. Suspensions were dispensed (0.2 mL) in to three test tubes, a nutrient agar slope (Oxoid, England) and a CLED (Oxoid, England) purity plate. Individual reagent tablets (Rosco, Denmark) were then added to the tubes and incubated at 37°°C/3 h. Results were compared with Table 1 for identification. The API rapid 20E gallery (bioMerieux, France) was used for full biochemical confirmation. Polyvalent and group specific sera (Murex, England) were used for serological identification. During a 6-month period 2500 stools were examined giving rise to 265 suspect organisms for testing. In addition 140 known stock organisms (119 *Salmonella* and 21 *Shigella* species) were tested. Biochemical reactions were controlled with *Salmonella typhimurium* ACTC 14028, Proteus mirabilis ACTC 14153 and *Escherichia coli* ACTC 10536.

Table 1 LOUIS test algorithm

LDC	ONPG	URE	IND	Possible identification	Step 1	Step 2	
+	+	−	+	*E. coli*	Discard		
+	−	−	+				
−	−	+	+	*Proteus* spp. or	Discard		
−	−	+	−	*Morganella morgani*			
+	−	−	−	*Salmonella*	Confirm by serology (O and H antigens)	Positive: API rapid 20E plus sensitivity	Negative serology. Discard
−	−	−	−	*Shigella* spp. (possible LDC negative *Salmonella*)	API rapid 20E (4 h)	Confirm with serology plus sensitivity	Negative serology. Discard
−	−	−	+	*Shigella* spp.	API rapid 20E (4 h)	Confirm with serology plus sensitivity	Negative serology. Discard
−	+	−	−	*Shigella sonnei* or *Sh. dysenteriae* 1	Confirm with serology	Positive: API rapid 20E plus sensitivity	Negative serology. Discard

+Discard any other recombination of reactions.

Results: Step 1 discarded 175 organisms (66%) as non-significant and correctly suggested 49 out of 50 *Salmonella* isolates (sensitivity 98%, specificity 98.6%). Step 2 discarded a further 29 organisms leaving 12 organisms for full biochemical identification (excluding the 49 serologically confirmed *Salmonella* isolates). Of these 12 identifications, only 2 were significant isolates (1 LDC negative *Salmonella* and 1 *Shigella*). Overall the LOUIS test showed a sensitivity of 100% and a specificity of 94%. Stock organisms were all correctly identified by the 2-step LOUIS test protocol. Two *Salmonella* isolates were LDC negative giving a sensitivity of 98%. Negative reports were confirmed after examination of the CLED purity plates (24 h).
Conclusion: Early presumptive reporting of *Salmonella* can be achieved with a positive LDC profile with confirmatory serology 3 h after isolation on primary media. This would be of real value in outbreak situations both nosocomial and in the community.

P931 The comparison of Cary-Blair and selenit containing Dio-transportswab-SST transport media

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Objectives: Cary-Blair is a classic transport medium which enhances the survival of enteric bacterial pathogens like *Salmonella* and *Shigella*. Dio-transportswab-SST (Diomed) is a newly developed transport medium with properties of Cary-Blair in addition to containing selenium salts to suppress the growth of enteric bacterial flora and facilitate the isolation of enteric pathogens like *Salmonella*. In this study, to detect the suitability of SST, the two transport media were compared.
Methods: The turbidities of *Salmonella typhi* ATCC 14028 and Shigella sonnei ATCC 25931 suspensions were adjusted to that of 0.5 McFarland standard (1.5×108 CFU/mL) and diluted 1:10 (1.5×107 CFU/mL) with saline. 100 μL from these dilutions were added to each transport medium, reaching a final inoculum of 106 CFU/mL. Transport media were incubated at room temperature and +4 °C. Serial dilutions (1:10, 1:100, 1:1000) were prepared from both transports and 100 μL from each dilution were double plated at 0, 6, 24 and 48 h. Bacterial count was measured by taking the average of the two plates. For detection of the clinical efficacy of the SST medium, 300 stool samples of diarrheal patients were examined. Each stool sample was taken both into Cary-Blair and SST. While samples from SST were plated directly onto McConkey agar, those from Cary-Blair were plated onto McConkey agar and to selenit F broth. After 8–12 h of incubation, selenit F broth was subcultured again onto McConkey. Lactose non-fermenting suspect colonies were screened biochemically and serologically for *Salmonella* and *Shigella*.
Results: The culture results of SST and Cary-Blair transport media were found similar. In addition, there were no differences between 24 and 48 h culture results of SST but keeping SST at room temperature seemed to be more appropriate. According to stool culture results; six *Salmonella* and nine *Shigella* spp. were isolated from both transport media. One *Salmonella* was isolated only from SST and another only from Cary-Blair.
Conclusion: For carrying *Salmonella* and *Shigella*, SST transport medium was found as effective as Cary-Blair. For SST, there is no need to use selenit F broth and extra incubation; it is easier, faster and more cost effective for both transport and culturing of fecal specimens.

P932 Comparison of three methods: agglutination test, Dot ELISA and culture of feces for diagnosis of inapparent infection of *Salmonella* in calves

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Objective: Diagnosis of *Salmonella* carrier calves by using feces culture, Agglutination test and dot ELISA.
Procedure: 216 sera and feces samples were obtained from calves in farms around Tehran. The feces samples were cultured in enrichment and selective media and then isolated *Salmonella* were serotyped by O and H antisera. The sera samples were tested for O and H agglutinins by Widal and dot Elisa tests.
Results: In this study two serotypes including: *Salmonella typhimurium* (four cases) and *Salmonella dublin* (two cases) were isolated from feces. In serological tests 5 and 15 sera samples were positive in Widal and Elisa tests, respectively.
Discussion: The results of this study showed that dot Elisa method is very sensitive in diagnosis of *Salmonella* carrier calves than other tests especially fecal culture.

P933 SM ID2, a new chromogenic medium for isolation, detection and presumptive identification of *Salmonella*: comparison with SM ID, Hektoen and four commercially available chromogenic media

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Objectives: SM ID2 (bioMérieux) is a new, ready-to-use chromogenic medium for selective isolation and differentiation of *Salmonella* in human stools. Detection of *Salmonella* is based on the expression of esterase activity revealed by pink-colored colonies. Non-*Salmonella* species produce blue, green or colorless colonies. The purpose of this study was to compare the performance of SM ID2 to that of SM ID, Hektoen and four commercially available chromogenic media.
Methods: SM ID2 was compared with SM ID, CHROMagar *Salmonella* (Becton Dickinson), ASAP (A.E.S Laboratoire), OSCM (Oxoid) and Rambach agar (Merck), and to the conventional Hektoen medium. A comparison was made using a collection of 126 strains: 50 *Salmonella*, including some atypical strains, and 76 microorganisms frequently encountered in stool specimens.
Results: After 24 h of incubation, fertility of *Salmonella* was higher with SM ID2, SM ID and Rambach agar (100%) than with Hektoen (98%), OSCM (96%), ASAP (88%) or CHROMagar *Salmonella* (84%). As for all the chromogenic media tested, the selectivity of SM ID2 against yeasts, Gram-positive strains and non-fermenting bacilli was high, whereas selectivity against Enterobacteriaceae was low. Sensitivity after 24 and 48 h of incubation was 84 and 86% with SM ID2, 86 and 92% with OSCM, 82 and 90% with Hektoen, 80 and 80% with SM ID, 76 and 60% with Rambach agar, 74 and 90% with ASAP, and 56 and 78% with CHROMagar *Salmonella*, respectively. The specificity after 24 and 48 h of incubation of all chromogenic media was greater than 84%, except for OSCM which showed 80 and 67%, respectively (72.4 and 67.1% with Hektoen). Out of all the highly specific chromogenic media (specificity greater than 92%), SM ID2 was the most sensitive medium.
Conclusion: The high specificity and sensitivity of SM ID2, after 24 h of incubation, makes it a chromogenic medium of choice for the detection and presumptive identification of *Salmonella* in stool samples.

P934 DIO-*Salmonella* selective medium, a novel medium for specific isolation of *Salmonella* spp.

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Objectives: DIO-SSM (Diomed, Turkey), is a new chromogenic medium for the isolation and presumptive identification of *Salmonella* spp. It is a clear pale yellow liquid medium that turns black when *Salmonella* grows in it. No other species tested so far including *Proteus* spp. has this effect. They either do not grow or create turbidity without color change.
Methods: DIO-SSM was evaluated with a total of 192 strains of *Salmonella* and standard strains, consisting of 13 reference strains and 179 clinical isolates which were previously all identified biochemically and verified serologically.
Results: All *Salmonella* strains changed the color of the medium from yellow to black, others either did not grow or just turned it to turbid.
Conclusions: Compared with SS medium (*Salmonella Shigella* medium), DiO-SSM is a more specific medium in isolation of *Salmonella* spp. since the colonies of other bacterial species like *Proteus* spp. may also appear black on SS medium. DiO-SSM is a very practical, specific medium for isolating *Salmonella* from various samples

P935 The potential diagnostic value of clinical and laboratory findings in typhoid fever

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Objectives: To evaluate the predictive value of various clinical and laboratory features in cases with culture-proven typhoid fever.
Methods: A retrospective analysis was done on hospital charts of culture-proven typhoid fever cases. The important associated features with typhoid fever as constipation, diarrhea, hepatomegaly, splenomegaly, discordance between pulse and fever, Widal test, leukopenia and an elevated aspartate

aminotransferase (AST) more than 1.5 times were evaluated for diagnostic predictive value on admission. A simple model to predict diagnosis of typhoid fever was built by using four features the most frequent associated.
Results: A total of 129 blood culture proven typhoid fever cases were evaluated using clinical and laboratory features. There were 66 males and 63 females. Average age was 23.7 ± 10.5 years. The mean preadmission duration of fever interval was 11.7 ± 8.8 days. The most frequent associated features with typhoid fever were discordance in 100 cases (77.5%), high AST in 95 (73.6%), Widal test in 66, leukopenia in 56 (43.4%), diarrhea in 44 (34.1%), splenomegaly in 37 (27.7%), hepatomegaly in 20 (15.5%) and constipation in 15 cases (11.6%). At least two of the most frequent associated features (discordance, high AST, Widal test, leukopenia) was found positive in 121 cases (93.8%). Two features were found positive in 50 cases (38.8%), three features in 50 cases (40.3%) and four features in 19 cases (14.7%).
Conclusions: The predictive value of these features for diagnosis of typhoid fever is found very high. The association of two or more features among discordance, high AST, Widal test and leukopenia may be used for early diagnose of suspecious typhoid fever cases.

P936 Characterization of anti-*Salmonella* monoclonal antibody (3D11)

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Background: The genus *Salmonella* is a member of Enterobacteriaceae and is composed of phenotypically and genotypically related bacteria. *Salmonella* are Gram-negative facultative anerobic rods. It causes many diseases in man e.g. typhoid fever, gasteroenteritis and food poisoning. For rapid and direct detection of bacteria 3D11 monoclonal antibody was developed against heat killed *Salmonella* strains in the Department of Immunology, Strathclyde University, Glasgow. The aim of this study was characterization of the antibody enable to be used as a diagnostic and therapeutic agent in clinical fields.
Material and methods: More than two hundred *Salmonella*, *E. coli* and other Gram-negative and gram positive bacteria were purchased from NCTC colindale, UK and many bacterial strains kindly donated by Stobhil Hospital, Glasgow. LPS, lipid A, outer membrane proteins, flagellar antigens, Ra mutant LPS were extracted from salmonella. The reactivity of antibody was assessed with all extracted materials using ELISA, SDS–PAGE, and Western blotting and dot blotting techniques.
Results: The antibody was reactive with only heat killed *Salmonella* strains. It is strongly reactive with polysaccharide, smooth LPS, and not reactive with polypeptide, phospholipids and flagellar antigens. More characterization and epitope mapping showed the antibody was developed against outer core of LPS.
Discussion: The results were indicated the epitope is not accessible to the antibody in live bacteria and when heated a strong positive reaction was observed. The heat treatment destroys the integrity of the outermembrane in bacterial strains. The results were indicated the outer core of LPS is conserved in all *Salmonella* strains and trissacharides are immunodominant sugars in all salmonellae strains. This antibody should be used as a diagnostic and therapeutic purposes.

P937 Evaluation of jackbean meal as a control for rapid urease tests and introduction of a new room temperature stable rapid urease test for diagnosis of *Helicobacter pylori*

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Objectives: To determine whether jackbean meal which contains urease can be used as a positive control for rapid urease tests and also to find out whether a paper-strip rapid urease test (RUTP) containing urea, phenol red and acidic salt which is stable at room temperature was reliable when compared with histology in the diagnosis of *Helicobacter pylori* infection.
Methods: Prospective consecutive sampling were done of 83 selected patients who underwent outpatient esophagogastroduodenoscopy and antral biopsies were taken. CLO test, RUTP, and histology examinations were done on obtained specimens. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the RUTP were calculated and compared. For standardization of RUTP, biopsy samples of another 179 patients were studied and analyzed. Jackbean meal was used as control for all CLO and RUTP tests.
Results: Amongst 83 patients, the sensitivity, specificity, positive and negative predictive values and accuracy of RUTP were 91, 100, 100, 100, and 95% which appeared very significant. RUTP was stable for more than 6 months at room temperature. Jackbean meal was also stable at room temperature and it was found as an ideal control for rapid urease tests.
Conclusions: RUTP is an inexpensive test stable at room temperature with good sensitivity and specificity for *H. pylori* infection and jackbean meal is an ideal control which may be used in all rapid urease test kits as there is no control in all presently available rapid urease test kits.

P938 Western blot analysis for the serological detection of CagA and VacA status in *Helicobacter pylori* seropositive children

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Objectives: The assessment of CagA and VacA seropositivity in children by the Western blot technique.
Methods: 59 dyspeptic children (M: 26, F: 33) aged 4–14 years, grouped by the presence of anti-*H. pylori* (HP) IgG or IgA antibodies (ELISA), were studied. Group I included 33 children IgG positives and IgA negatives, Group II 19 children IgG and IgA positives and group III 7 children IgA positives and IgG negatives. All groups were tested for CagA and VacA specific IgG or IgA antibodies with Western blot analysis.
Results: In the first group, 36.4 and 24.2% of the children presented reactivity against the CagA and the VacA band, respectively. In the third group, 28.6% of the children were CagA positives, but none VacA positive. In the second group, IgG antibodies against CagA and VacA antigen observed in 100 and 94.1% of the children, and IgA antibodies in 68.4 and 52.6%. IgG antibody response to CagA and to VacA was expressed in 56.9 and 47.1% of the IgG seropositive children (Groups I and II) and IgA response in 57.7 and 38.5% of the IgA seropositive children (Groups II and III). We found that CagA or VacA seropositivity was associated with statistically significant ($P < 0.05$) higher anti-HP ELISA titers.
Conclusions: Detection of specific antibodies against CagA and VacA antigen by Western blot, may be useful in the screening of severe *H. pylori* infection.

P939 Simultaneous assessment of *H. Pylori* (by means of cultural and rapid urease test) using a newly developed transport medium

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Background: Culture is one of the most important diagnostic tools for assessment of *H. pylori* infection before and after therapy.
Obiective: To evaluate the diagnostic value of new vials (Pyloriset(R) urease, Orion Diagnostica) for rapid assessment of HP infection and culture in comparison with rapid urease test (CP test) and standard culture technique.
Materials and methods: Gastric biopsies from a total number of 115 patients who underwent gastroscopy for dyspeptic symptoms, were taken. All subjects had not received antibiotics during the last three months before endoscopy. During gastroscopy, five biopsies were taken from antrum (three biopsies) and corpus (two biopsies) and used (one biopsy) for rapid urease test (CP test), standard culture with refrigerated NaCl 0.9% as transport medium (two biopsies), and two biopsies were put in the new vials containing new medium (Pyloriset(R) urease) for rapid HP diagnosis and subsequent culture. Results from culture perfomed from biopsies transported with the standard medium in NaCl 0.9% were compared with results of CP test at 30 and 120 min, with rapid HP test from biopsies put into Pyloriset(R) vials, and with culture with Pyloriset(R) urease as transport medium.
Results: See Table 1.

Table 1

Test	Sensibility (%)	Specificity (%)	PPV (%)	NPV (%)	Concordance (%)
Culture Orion	93	100	100	85	95
CP 30	85	85	93	71	80
CP 120	86	85	93	73	83
Orion 30	88	80	90	76	79
Orion 120	88	80	90	76	79

Conclusions: (1) Cultures performed from vials containing Pyloriset(R) urease shown high rates of sensibility and specificity and are therefore comparable with standard culture technique; (2) The result of the rapid HP test using the new vials are similar to those obtained with CP test; (3) Sensibility of Pyloriset(R) rapid urease test was already high at 30 min and similar to the one at 120 min; (4) Bioptic assessment of HP by means of rapid test and culture with Pyloriset(R) urease represents therefore a promising technical advance.

P940 Determination of the levels of procalcitonin and C-reactive protein in the diagnosis of *H. pylori* infections and the value of these markers in the post-treatment follow-up of *H. pylori* eradication

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Objectives: To determine, the levels of PCT and CRP in *Helicobacter pylori* (+) cases diagnosed as duodenal and gastric ulcer and to evaluate the correlation of PCT and CRP with other invasive and noninvasive methods in *H. pylori* eradication in the post-treatment follow-up.
Methods: Forty patients with dyspepsia were included in this study. 5 mL serum samples were collected in admission and after 24 h. One week antimicrobial therapy (omeprazol, amoxicillin and clarithromycin) was given to the HP(+) patients with the result of only culture or urease + pathology. 5 mL serum samples were collected again and culture, urease, pathology investigations were performed for endoscopic samples after one month. PCT and CRP levels were measured in the collected blood samples. 35 HP(+) cases with peptic ulcer and 38 cases with bacteremia and 38 healthy blood donors were included in this study as control groups.
Results: The mean of PCT and levels (minimum-median-maximum), were found as 1.39 (0.25–0.73–6.75), 0.35 (0.12–0.35–0.71), 7.45 (0.68–4.51–51.5), 0.40 (0.12–0.41–0.71) for the groups of HP (+), HP (–), bacteremia and healthy donors, respectively. The mean of CRP levels were found as 1.00 (<0.5–<0.5–8.11), 0.62 (<0.5–<0.5–3.2), 11.5 (3.2–7.77–43.5), 0.63 (<0.5–<0.5–5.46) for the same groups. Between HR (+) cases and three other groups, it was found statistically difference for PCT levels in admission ($P < 0.05$). It was observed a statistically decrease in PCT levels between the admission and post-treatment period ($P < 0.05$).
Conclusion: HP(+) cases were found to have lesser PCT levels than bacteremia cases in admission. A statistically significant decrease was observed in PCT levels after the post-treatment period against the PCT levels in admission. In contrast to this observation CRP levels were found in similar range, both in admission and after the post-treatment period. Sensitivity of PCT was found higher but specificity of PCT was found lower against CRP in admission. PCT and CRP, were found to have similar sensitivity and specificity levels after treatment.

P941 Serum anti-CagA antibodies in patients with different clinical symptoms of *Helicobacter pylori* infection

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Objectives: To estimate the frequenty of anti-CagA antobodies in the serum of patients with *H. pylori* infection and having different symptons of diseases.
Methods: The studies were performed on 78 serums obtained from adult patients (mean age 48 years).
More specifically: Thirty-nine serums came from patients with duodenal ulcer, 22 with gastritis, 3 with gastric cancer and 14 with rosaceae. Serum anti-CagA antibodies of IgG and IgA classes were detected by Helicobacter p120 (CagA) ELISA test (VIVA Diagnostica). The presence of *H. pylori* in gastric biopsies was detected by culture method, using Columbia-agar with 7% sheep blood and antibiotics. The samples were incubated under microaerophilic conditions for 4–10 days.
Results: Thirty-five (90%) patients with duodenal ulcer demonstrated the presence of anti-CagA antibodies of IgG class and 29 (74%) of IgA class. All patients with gastric cancer showed the presence of anti-CagA antibodies of IgG class only. In the group of patients with rosaceae—6 of them (43%) had anti-CagA antibodies, of both IgG and IgA class. Only four patients (18%) with gastritis demonstrated the presence of anti-CagA antibodies of IgG class and three of them (13%) of IgA class.
Conclusion: Our results indicate a relationship between anti-CagA seropositivity and an increasing risk for the development of more than dangerous clinical forms of *H. pylori* infection.

P942 3-Polymerase chain reaction assay for the detection of *Helicobacter pylori* in gastric tumor specimens: comparison with histopathologic studies

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Helicobacter pylori, particularly the cag A positive strain, has been associated with gastric tumors. The aim of the present study was to evaluate the frequency of cag A positive strain of *H. pylori* in cases of gastric tumors by PCR assay, and to correlate between *H. pylori* and the patients' age, sex and tumor istologic subtypes and grades. Thirty gastric tumor patients (male to female ratio: 2:1 mean age: 55.5 years; age range: 28–75 years) and 20 control cases were studied. The presence of the *ure C* gene which is indicative of *H. pylori* infection, and the cag A genotype were determined by PCR assay. *ure C* gene was detected in 70 and 40% of the gastric tumor and control cases, respectively. *cag A* gene was detected in 63.3 and 20% of the gastric tumor and control cases, respectively. 90.5% of *H. pylori* strains detected in gastric tumor patients were cag A positive compared with 50% in the control group. In conclusion, there is a significant association between *H. pylori* cag A positive type infection and gastric tumors.

P943 *Helicobacter pylori* antigen investigation in stool specimens with ELISA method

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Objectives: Many invasive and non-invasive methods are used in the diagnosis of *Helicobacter pylori* infections. All of the tests have specific properties, but a test with high sensitivity and specificity to be easily applicable and also economic was not defined yet. Recently, a new non-invasive test was improved for the detection of *H. pylori* antigen with ELISA method in stool specimens. In this study, the applicability of *H. pylori* antigen test (Genesis Diagnostics Ltd., United Kingdom) for *H. pylori* infections was evaluated by using sensitivity and specificity, positive predictive values(PPV) and negative predictive values(NPV).
Methods: A total of 62 patients (age average: 42.6) 33 of male and 29 of women who had gastrointestinal system endoscopy with the complaint of dyspepsia were included in this study. While selecting these patients, antimicrobial therapy, proton pumb inhibitors (PPI) and bismute compounds were not taken into consideration. A total of four biopsy specimens, one from corpus and three from the antrum of the stomach were obtained during the gastrointestinal system endoscopy from all of the patients. Biopsy specimens were investigated for rapid urease test, culture and histopathologic examination. *H. pylori* antigens were investigated from the the simultaneously collected stool specimens from all of the patients with the ELISA method.
Results: Patients were defined as *H. pylori* positive when histopathologic examination and urease test or only culture were positive. The other patients' test result were classified as *H. pylori* negative. 43 (69.4%) and 19 (30.6%) of 62 patients were found as *H. pylori* positive and *H. pylori* negative, respectively. We compared the results of *H. pylori* positive and *H. pylori* negative patients with the test results of *H. pylori* antigen test. We found the sensitivity and specificity, PPV, NPV, of *H. pylori* antigen test as 83.7, 52.6, 80 and 58.8%, respectively, with our test results.
Conclusions: *H. pylori* antigen test is not found sufficient only in the light of our findings for the diagnosis of *H. pylori* infection.

P944 Evaluation of two commercial culture media for the isolation of toxigenic *Clostridium difficile* from fecal samples

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Background: *Clostridium difficile*-associated diarrhea is a very common nosocomial infection that contributes significantly to patient morbidity and mortality as well as to the cost of hospitalization. Diagnosis of *C. difficile*-associated diarrhea is based in the toxin detection by a tissue culture cytotoxin assay. Isolation of *C. difficile* is performed in certain institutions with diagnostic and epidemiologic purposes.
Objectives: To evaluate two commercial culture media, one containing blood (*C. difficile* agar, CDA, bioMerieux®) and the other without blood (CCFA, Difco®), for the isolation of *C. difficile* in human fecal samples for patients suspected of having *C. difficile*-associated diarrhea.
Methods: A total of 96 consecutive fecal specimens were cultured in both culture media and processed by the tissue culture assay. For comparison purposes, the combination of the two culture media and the tissue culture assay performed directly from the feces or from the isolate ('second look') was considered the gold standard. The sensitivity value of both types of culture media was compared using the exact binomial test for paired samples.
Results: Overall, 23 specimens (24%) yielded toxigenic *C. difficile* isolates (TCD), and only one (1%) yielded a nontoxigenic isolates (NTCD). CDA media recovered all the 23 TCD and the NTCD, while CCFA only recovered 17 CDT. In four of the 22 samples, the direct tissue culture assay showed false negative results giving a positive result only when the 'second look' was performed. Moreover, the sensitivity values for CDA and CCFA media were, respectively, 100 versus 73.9 ($P = 0.03$).
Conclusions: Our findings show that CDA media was significantly more sensitive than CCFA media for the isolation of *C. difficile*. Combination of a sensitive culture media with the tissue culture assay increases the diagnostic yield of *C. difficile*-associated diarrhea.

P945 Heat-stable Enterotoxin producing Enterobacteriacae isolated from diarrheal children in Iran

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Objectives: Certain members of Enterobacteriacae elaborate heat-stable enterotoxins (ST) which contribute to their virulence. Diarrheal diseases are a major cause of morbidity and mortality of infants and children in developing countries. In Iran, they constitute a major public health concern.
Methods: To survey the prevalence of ST secreting enteric bacteria in diarrheal children, 250 stool samples from infants and kids suffering from watery diarrhea as well as, 50 stool samples from nondiarrheal children as a control group with the same relative age, sex, and family socio-economic status were studied. Following stool culture and isolation of the presumed pathogenic bacteria, their ability to secrete ST was examined by suckling mouse assay.
Results: From a total of 148 bacterial strains isolated from the diarrheal samples (*Escherichia*, *Citrobacter*, *Klebsiella*, *Yersinia*, and *Enterobacter* spp.), 35 of them were able to produce detectable levels of ST (14.3%). Whereas, only one strain from the control group (an *E. coli* isolate) was ST positive (2%). Most ST producing bacteria were isolated from children with less than 3 years of age.
Conclusion: The statistically significant difference in the prevalence of ST positive bacteria in the diarrheal versus control group establishes a clear involvement of the enterotoxin in the etiology of watery diarrhea.

Fungal infection: clinical epidemiology

P946 Epidemiology and susceptibility of *Candida* isolates from high and low risk patients in Denmark to eight antifungal compounds

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Objectives: An expected consequence of the increase in number and use of antifungal compounds is a selection of fungal isolates with decreased susceptibility. We conducted a study of the susceptibility of *Candida* isolates obtained from patients under low and high selective pressure, respectively.
Methods: A total of 233 *Candida* isolates were studied. 185 were blood stream isolates collected in the period 1994–2001 from the Copenhagen County. The remaining isolates were from patients at an intensive care unit in 2002. MIC's of eight antifungal agents were determined following the NCCLS microbroth-dilution method.
Results: The annual incidence of candidemia increased from 1.8 to 4.9 per 100 000 of the population in the observation period. All isolates were susceptible to amphotericin B (MIC's ≤ 1 μg/mL) and caspofungin (MIC's ≤ 2 μg/mL). Combined fluconazole, ketoconazole, itraconazole and voriconazole resistance was observed for 3/37 *C. albicans* isolates obtained from high-risk patients while no such isolates were found from the low-risk patients. Also a difference in MIC50 and MIC90 for fluconazole was demonstrated among low and high-risk patients: MIC50 0.5 versus 1 and MIC90 1 versus 8 μg/mL, respectively ($P = 0.018$). *C. glabrata* and *C. krusei* isolates were all voriconazole susceptible (MIC's of ≤ 2 μg/mL). With the exception of *C. parapsilosis* isolates the MIC50 of terbinafine was ≥ 16 for all species. Resistance to flucytocine was demonstrated among all *Candida* species with the exception of *C. glabrata* and *C. parapsilosis*. In conclusion, isolates from patients under high selective pressure were found to be more azole resistant than isolates from low risk area. This resistance included voriconazole although the drug has not yet been introduced in Denmark.

P947 Recovery of *Candida dubliniensis* from nonhuman immunodeficiency virus-infected patients in Kuwait and its rapid identification using seminested PCR amplification of rDNA

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Objective: *Candida dubliniensis* is an emerging pathogen capable of causing oropharyngeal, vaginal and bloodstream infections. The aim of this study was to isolate *C. dubliniensis* from clinical specimens and to develop a rapid molecular identification method by exploiting its genotypic differences with *C. albicans*.
Methods: Four clinical isolates of *C. dubliniensis* were recovered from immunosuppressed patients attending various hospitals in Kuwait. The molecular test to identify *C. dubliniensis* was based on amplification of high copy rDNA. The universal fungal outer primers amplified the 3′ end of 5.8S and 5′end of 28S rDNA including the internally transcribed spacer 2 (ITS2). The species-specific primer was derived from ITS2 sequence of *C. dubliniensis* and was used together with outer universal reverse primer in the re-amplification step of the snPCR.
Results: We describe the first four isolations of *C. dubliniensis* from Kuwait, all originating from nonhuman immunodeficiency virus (HIV)-infected patients. The isolates were initially identified by Vitek 2 yeast identification system and subsequently confirmed by positive germ tube test, production of chlamydospores on Staib agar, and by their inability to assimilate xylose and α-methyl-D-glucoside. The species-specific primer corresponding to unique sequences within ITS2 of *C. dubliniensis*, together with universal fungal primer amplified DNA from *C. dubliniensis* reference strain only and not from *C. albicans* or other *Candida* species in the re-amplification step of the snPCR. The snPCR identified all the four clinical isolates as *C. dubliniensis*. The identity of our clinical isolates was confirmed by further molecular characterization and direct DNA sequencing of ITS2.

Conclusions: The isolation of *C. dubliniensis* from four non-HIV-infected patients from Kuwait reinforces the existing view that this novel yeast species has a worldwide distribution and its occurrence is not restricted to any particular immunocompromised population. The snPCR developed in this study is rapid, specific and more sensitive than biochemical methods and other PCR-based methods for detecting and differentiating *C. dubliniensis* from *C. albicans* in clinical specimens.
Supported by Research Administration projects grants MI 118, Kuwait University.

P948 Identification, clinical and epidemiologic aspects in candidiasis

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Background: Fungal infection remains a common health problem. The aim of this study has been to determine the incidence and species distribution of infectious agents for 228 hospitalized patients for clinical assumption of fungal infection.
Methods: The laboratory diagnosis has been established by bacteriologic and mycologic examinations: direct microscopic examination of patient samples in a drop of lactic blue or 30% potassium hydroxide, culture on Sabouraud's agar and identification of the isolated *Candida* strains by biochemical tests.
Results: *Candida* spp. has been isolated from patient's samples. The most common isolated species have been *C. albicans* (65%) followed by *C. krusei* (21%), *C. glabrata* (14%). Mucosal candidiasis prevailed (73.1%) followed by cutaneous candidiasis (25.2%) and candidemia (1.8%). The prevalent mucosal candidiasis included: enterocolitis (43.5%), vaginitis (13.6%), oral candidiasis (12.4%). The prevalent cutaneous candidiasis included eczema (14.2%) and onychomycosis (2.4%). Prolonged antibiotic therapy favored the occurrence of glossitis, enterocolitis and vaginitis. Diabetes mellitus represented a predisposing factor for cutaneous, vaginal and urethral candidiasis while pregnancy represented a predisposing factor for vaginitis. Candidiasis occurred both in children (50.3%) and in adults (49.7%) but there have been differences in location. In children the most common were enteritis and colitis (54.7%), followed by glossitis (18.8%), intertrigo (17.6%), cystitis and urethritis (5.09%), candidemia (2.9%). In adults we found similar proportions of enterocolitis (32.1%), cutaneous candidiasis (32.7%) and urogenital candidiasis (28.6%) followed by less frequent glossitis (6%) and candidemia (0.6%). Candidiasis had a slightly higher incidence in women (55.1%) then in men (44.9%), vaginal (79.5%) and oral (60%) candidiasis being prevalent.
Conclusions: Knowledge of clinical manifestations and predisposing factors is profitable due to correlations that can be made between different candidiasis and *Candida* spp., patient state, age and sex, in order to establish the proper prophylaxis and treatment. An important issue in therapy of fungal infections is the occurrence of resistant strains, both to common antifungal drugs and to newly discovered ones. It is important to study the susceptibility of *Candida* spp. to antifungal drugs in order to effectively fight these infections.

P949 Species distribution and antifungal susceptibility of yeasts and molds isolated at Cerrahpasa Medical Faculty Department of Microbiology and Clinical Microbiology Deep Mycoses Laboratory between 02 January and 27 December 2002

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The aim of this study is to characterize the species distribution and antifungal susceptibility patterns of yeast and mold strains isolated at a Turkish University Hospital deep mycoses laboratory. Totally 300 specimens belonging to various body sites (10 CSF, 4 corneal, 4 ear, 9 nose, 2 nasopharinx, 72 sputum, 3 ETA, 89 BAL, 8 pleural, 13 oral, 6 gastric aspiration, 2 feces, 13 urea, 2 vaginal, 1 pericardium, 2 hemoculture, 28 blood, 1 articular fluid, 7 pus, 4 bone marrow, 16 tissue biopsy specimens) submitted from different units of the hospital were studied. Yeast and mold strains isolated were identified according to classical mycologic methods. Antifungal susceptibility pattern of the clinically significant yeast and mold isolates recovered from deep mycoses suspected patients' specimens over 1-year period determined against conventional agents using the US National Committee for Clinical Laboratory Standards M27-A and M38-P reference methods. A total of 54 yeasts and 11 molds strains were isolated. *Candida albicans* strains were 14.8% (8/54), nonalbicans *Candida* species were 81.5% (44/54), *Trichosporon* sp. 1.9% (1/54), and *Cryptococcus neoformans* 1.9% (1/54). The molds isolated were *Aspergillus* spp. 57.1% (8/14), *Paecilomyces* spp. 21.4% (3/14), *Fusarium oxysporum* 7.1% (1/14) and 2 hyphomicetes fungi 14.3% (2/14) (identification is continuing). No growth was observed in two antifungal receiving patients' materials inspite of direct microscopical positivity of fungal elements. In a case with aspergillosis clinical diagnosis due to the symptoms and radiological findings, *Paecilomyces* sp. was recovered from BAL specimen and *Aspergillus* latex agglutination test was found negative. *F. oxysporum* was isolated from a case of keratitis. In vitro resistance to fluconazole (MIC higher than or equal to 64 micrograms per milliliters) as per NCCLS criteria was observed in 2 *C. albicans* (25%), 13 nonalbicans *Candida* strains (29.5%), resistance to itraconazole (MIC higher than or equal to 16 micrograms per mililiters) in 1 *Candida* (12.5%), 9 nonalbicans *Candida* (20.5%). All *Aspergillus niger* strains showed high MIC values to both azoles. *F. oxysporum* isolate was found in vitro resistant to all antifungals tested. We observed that nonalbicans *Candida* spp. and *Aspergillus* tend to show again high prevalence along with the presence of rare opportunistic molds other than *Aspergillus* as were in the past three years findings of our deep mycosis laboratory.

P950 Oral candidosis, new perspective: mixtures of *Candida* spp. and antifungal therapy

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Objectives: The purpose of this study was to prospect for mixed cultures of *Candida* spp. in 53 oral samples and to assess their antifungal susceptibilities with respect to a possible relapse of infection due to proliferation of the coexistent species usually not considered in routine laboratory practice.
Materials and methods: 53 oropharyngeal samples were included in the study. For identification, the colony appearance on Chromagar *Candida* medium, chlamidospores production and substrate assimilation profiles with API 20 C aux system were used. The fluconazole (FL) susceptibility assessment was conducted using *E*-test method and ATB Fungus (BioMérieux, France), was used to assess for susceptibility to six antifungals: nistatine (NIS), miconazole (MIC), econazole (ECO), ketoconazole (KET), amphotericine B (AMB) and 5-flucitosyne (5FC).
Results: 8 from 53 samples (15%) represented mixed cultures (4 double, 4 triple). Colony numbering on primary isolation plate was used to consider the species causing the infection and coexistent species as well. In these samples the dominant species were *Candida albicans* in 7 cases and *Candida glabrata* in 1 case. In 50% of samples, the coexistent species (*C. krusei*, *C. norvegensis*, *C. lusitaniae*) were more resistant to antifungal agents than *C. albicans*. By chance, in one sample, we recovered two different strains of *C. albicans* which were identified by means of susceptibility patterns for antifungals.
All 20 isolates presented distinct susceptibility patterns for the 7 antifungal agents tested.
Conclusions:

1. Two different strains of *C. albicans* with major susceptibility patterns discrepancies could coexist in one sample.
2. *C. albicans* is not always the main species in a mixture of yeasts from oral samples; it could play a secondary role.
3. Species of *Candida* less susceptible could be easily selected after antifungal treatment if the coexisting species are not considered.
4. Antifungal therapy, according to susceptibility patterns of all *Candida* isolates in one sample could be the key for increasing incidence of non albicans species of *Candida*.
5. In the light of our results, the use of higher doses of Fluconazole could avoid the selection problem.

P951 The prevalence of vulvovaginal candidosis, bacterial vaginosis and *Trichomonas vaginalis* infection in patients with vulvovaginitis

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Objective: The purpose of this study was to detect the prevalence of vulvovaginal candidosis *Trichomonas vaginalis* and bacterial vaginosis in patients with diagnosis of vulvovaginitis.

Material and methods: A total of 100 nonpregnant, nondiabetic patients, ages between 25 and 35, applied to Obstetrics and Gynecology Clinic of Kocaeli University were enrolled to this study. Vaginal secretions from patients with clinical findings were investigated by direct and gram stained microscobic examination. All specimens were cultured into Sabouraud agar. Isolated *Candida* species were identified by germ tube test, morphological characteristics on cornmeal Tween 80 agar and carbohydrate fermentation tests. Bacterial vaginosis (BV) was diagnosed evaluating gram stained preparations according to Nugent's criteria, i.e. scores of 7 or higher was accepted as BV. *T. vaginalis* infection was diagnosed investigating direct and gram stained preparations. All patients were questioned about antibiotic or corticosteroid treatment, type of contraceptive method used, history of sexually transmitted diseases and the frequency of coitus.
Results: Totally 28 *Candida* species were isolated. They were identified as *C. albicans* (21, 82.1%), *C. glabrata* (4, 14.2%) *C. tropicalis* (2, 7.1%) and *C. krusei* (1, 3.4%). Only 84 preparation could be examined, and BV was detected in 32 patients (35.6%). In 11 patients (35.7%) with vulvovaginal candidosis (VVC), BV was also present. *T. vaginalis* was detected in 9 (10.6%) patients and none of them had accompanying candidosis. Demographic data of patients were evaluated for only patients with VVC. Two (7.1%) and three (10.7%) of them were using antibiotics and corticosteroids, respectively. Contraceptive methods used and the number of patients were as follows: IUD and condom (4, 14.2%), oral contraceptive and tube ligation (2; 7.1%), injection (1, 3.5%) and coitus interruptus (9, 30.2%). The frequency of coitus was once or twice a week (24, 80.5%) and once or twice a month (4, 14.2%). and two (7.1%) patients had history of previous VVC.
Conclusions: The most common pathogen causing VVC was detected as *C. albicans*. Since the high prevalence of BV in those patients was determined, patients with BV should be followed carefully and regularly for the possible presence of VVC, and vice versa. The high frequency of coitus could be related with VVC.

P952 Prevalence and sensitivity profile to 5 antifungal agents of yeast species causing onychomycosis and vaginitis

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Objectives: The present study was undertaken to investigate the yeast species most involved in onychomycosis and vaginitis and their in vitro sensitivity against amphotericin-B (AMP), 5-flucytosine (FLC), ketoconazole (KET), fluconazole (FLU) and itraconazole (ITRA).
Methods: Yeasts were collected from specimens obtained from 25 patients suffering from onychomycosis and 59 afflicted by vaginitis. Clinical samples were cultured on Chromalbicans Agar (Biolife). The fungal isolates were identified by using the YBC card (Vitek System bioMérieux) and confirmed by a commercial yeast identification kit API-*Candida* (bioMérieux). To evaluate the antifungal susceptibility, the broth microdilution test YeastOne (Sensititre) and the *E*-test (AB Biodisk) on RPMI 1640 glucose agar plate were used.
Results: The identification of isolates revealed the prevalence in vaginitis of *C. albicans* (52.3%), followed by *C. glabrata* (24.6%), *C. krusei* (4.6%), *C. parapsilosis* and *S. cerevisiae* (4.6%), while in onychomycosis the most frequent specie was *C. parapsilosis* (44%) followed by *C. albicans* (32%), *C. guilliermondii*, *C. lusitaniae*, *C. rugosa* and *C. terreus*. In vitro testing showed that the sensibility against FLU resulted high in vaginitis (75.4% of isolates) especially for *C. albicans* (91%); while the nonalbicans species were mostly sensible to AMP and to FLC, but showed higher levels of resistance to FLU. Indeed the 6.25% of *C. glabrata* isolates resulted resistant to this agent. In onychomycosis we observed intermediate levels of resistance to ITRA and KET in two *C. parapsilosis*, one *C. guilliermondii* and one *C. lusitaniae* strains. One *C. albicans* strain was resistant to AMP.
Conclusions: The results demonstrate an higher frequency of *C. parapsilosis* isolation compared with *C. albicans* in onychomycosis instead of in vaginitis. However, *C. glabrata* and other nonalbicans species characterized by broad resistance to antifungal agents are emerging as important causes of vaginitis. Thus, further studies are required to establish the possible role of these species in recurrent vaginal candidiasis.

P953 Causative agents of fungal nail infections in Russia: new species and implications for therapy

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Background: Little concordance is observed in modern views on etiology of fungal nail infection (onychomycosis). While some authors claim significant role of nondermatophyte molds and yeasts, others totally reject the right of nondermatophytes to cause infection.
Objectives: To examine current etiologic composition of onychomycosis in Russia, by prevalent genera and species, and also by major etiology groups.
Methods: Laboratory protocols (mycology unit of Central clinical hospital under Presidential medical center) for 1997–2001 years were analyzed. Primary material was collected from 5 hospitals and 2 clinics of Moscow region. During this period, there were cultured 3075 fungal isolates from 12617 samples.
Results: Dermatophytes (*Trichophyton*, *Epidermophyton* or *Microsporum* species) were isolated in 73.3%; *Candida* species in 9.6% and molds in 16.9%. Among fungi identified by genus and/or species *T. rubrum* was the leader (71.06%), followed by *Candida* spp. (8.8%) and *T. mentagrophytes* var. *interdigitale* (4.6%). Occurrence of different species changed from time to time. Among dermatophytes, *T. rubrum* took 93.3% and *T. mentagrophytes* was isolated 15 times less frequent with prominent variability in time. We have isolated 5 cultures of *T. mentagrophytes* from fingernails (1.8%). *M. canis*, *T. tonsurans* and *T. violaceum* onychomycosis was sporadic (12 cases total). Fungi were isolated from toenails 4–15 times more frequent, but positive fingerail cultures were increasing in number since 1997. Among fingernail isolates, proportion of dermatophytes was 38.4%, *Candida* spp. – 44.8% and molds – 16.5%. Among toenail isolates, proportion of dermatophytes was 79.9%, *Candida* spp. – 14.3%, molds – 14.3%. Among nondermatophyte mold fungi *Aspergillus* spp. and *Scopulariopsis brevicaulis* shared the same proportion of 38.3% each. Other species, such as *Acremonium*, *Alternaria* and *Fusarium* spp. had unique occurrence (although the majority of mold isolates except *Aspergillus* and *Scopulariosis* was not identified). We have described also two cases of onychomycosis caused by relatively rare *Aspergillus* species: *Aspergillus ustus* and *Aspergillus clavatus*.
Conclusions: In certain clinical patterns of onychomycoses various fungi may become a causative agent. This should point the clinician towards selective usage of antifungals of known spectrum or perform susceptibility testing.

P954 The incidence of opportunistic fungi in patients suspected of tuberculosis

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Objectives: Opportunistic fungal infections are one of the major mortality and morbidity in the debilitated patients. The patients affected with tuberculosis are among the immunocompromised hosts predispose to opportunistic fungal infections. In addition, pulmonary fungal infections manifest clinical syndromes which mimic tuberculosis. The objective of this study is to examine the incidence of opportunistic fungi in bronchoalveolar lavage (BAL) specimens of patients suspected of tuberculosis and to evaluate the significance of the findings.
Methods: The BAL specimens from 200 patients suspected of tuberculosis were collected by standard bronchoscopic procedure and examined by direct and culture methods for both tubercle bacilli and fungi.
Results: From 200 patients 36 yeasts (18%) and seven filamentous fungi (3.5%) were isolated and identified as follows: *Candida albicans* (26), *C. glabrata* (3), *C. tropicalis* (3), *C. pseudotropicalis* (2), *C. krusei* (1), *Rhodotorula rubra* (1), *Aspergillus fumigatus* (4), *A. flavus* (2), *A. niger* (1) and *Streptomyces griseus* one case. Out of 200 cases, 16 patients were affected with definite tuberculosis and 11 of these cases had pulmonary fungal infection in addition to tuberculosis.
Conclusion: From this study we can conclude that the yeasts had the highest frequencies and among filamentous fungi *A. fumigatus* was more frequently responsible for chronic pulmonary infections in patients suspected of tuberculosis in Iran. Therefore, attention should be paid to the recognition of opportunistic fungi isolated from patients presumed to have tuberculosis and that any organism should be considered a potential agent of infection in debilitated patients.

P955 Molecular epidemiology of *Candida* spp. isolated from urine at an intensive care unit, Turkey

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Objective: *Candida* spp. has been the leading microorganism isolated from the urine specimens of patients hospitalized at the Anesthesiology and Reanimation Intensive Care Unit (ICU) of Dokuz Eylul University Hospital, Izmir since 1998. This study was undertaken to investigate the clonal relationship of *Candida* urine isolates in order to find the mode of spread among the patients.
Methods: Epidemiologic surveillance of 38 *C. albicans*, 15 *C. tropicalis* and 10 *C. glabrata* recovered from the urine specimens of patients who were hospitalized in the ICU between June 11th 2000 and October 15th 2001 was done by antifungal susceptibility testing and randomly amplified polymorphic DNA (RAPD) analysis. Two short primers [Cnd3 (5′-CCAGATGCAC-3′) and Cnd4 (5′-ACGGTACACT-3′)] were used for RAPD.
Results: None of the isolates were resistant to amphotericin B with MIC50 values of 0.5 μg/mL, 0.5 μg/mL and 0.125 μg/mL for *C. albicans*, *C. tropicalis* and *C. glabrata* isolates, respectively. On the other hand, three *C. glabrata* isolates were resistant and one *C. albicans* and five *C. glabrata* isolates were dose-dependent susceptible to fluconazole. Among *C. albicans* isolates 22 and 24 patterns were detected with primers Cnd3 and Cnd4, respectively. Primer Cnd3 yielded four and primer Cnd4 yielded two patterns for *C. tropicalis* isolates. Among *C. glabrata* isolates four and five patterns were observed with the two primers.
Conclusion: Our results suggest that the source of *C. albicans* isolates were mostly endogenous. On the other hand, cross transmission among patients was considered for *C. tropicalis* and *C. glabrata*, as isolates that were clonally related were recovered from different patients hospitalized at the same time interval.

P956 The identification of *Candida* spp. isolated from different clinical samples and investigation of their susceptibility to antifungals

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Objective: At Haseki Education and Research Hospital microbiology laboratory, the identification and antifungal susceptibilities of *Candida* spp. isolated as an infectious agent from specimens were investigated.
Methods: 53 *Candida* spp. were isolated from urine, blood, sputum, abscess, ear discharge and vaginal secretions. These strains were identified according to their microscopic morphology, germ tube test and with API ID32C (bioMérieux, France). Their antifungal susceptibilities were detected by API ATB Fungus (bioMérieux, France).
Result: 25 strains were isolated from urine, 13 from blood, 8 from sputum and 7 from the others. If the strains are distributed according to their species, *C. albicans* was the majority (40). and it follows as *C. krusei* (5), *C. utilis* (3) and the others. Antifungal resistance was15% to flucytosine, 41.5% to amphotericin B, 39.6% to nystatin, 33.9% to miconazole, econazole and ketoconazole.
Conclusion: The resistance of *C. albicans* strains to ketoconazole was found to be 40% whereas it was found to be 19% in a study of ours in the year 1999. The identification of *Candida* spp. and to determine their antifungal susceptibilities will be a guide to the appropriate treatment of *Candida* infections.

Molecular diagnosis of fungi

P957 Rapid and specific detection of secreted aspartyl proteinases (SAP) genes of *Candida albicans* by LightCycler PCR

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Objectives: Saps are putative virulence factors in *Candida* infections. It is reported the sequences of 10 members of a SAP gene family in *Candida albicans* (SAP1–SAP10). The conventional PCR protocols for detection these genes are time- and labor-intensive. The LightCyclerTM (Roche) combines rapid amplification of nucleic acids in glass capillaries with melting curve analysis based on fluorescence resonance energy transfer (FRET) for the sequence-specific detection of the amplicons. Based on an established PCR protocol for the sensitive and specific detection of SAP genes, the present study describes the development and evaluation of that methodology for the detection of these genes.
Methods: 20 cultured strains were screened (10 *C. albicans*, 5 *C. parapsilosis*, 5 *C. tropicalis*), isolated from pediatric patients, and a reference strain, *C. albicans* SC5314, for the presence of defined amplicons by comparing conventional cycling with a LightCycler assay. One primer pair each for SAP1, SAP2, SAP3, SAP4, SAP6, SAP7, and SAP8, was chosen. After LightCycler run, gel electrophoresis was performed to have an independent validation check of the presence of an amplicon.
Results: All fragments from the reference and the other *C. albicans* strains were amplified and detected, whereas none of the fragments from other *Candida* species examined were recognized. The curves generated by the LightCycler software allowed for a clear discrimination between positive and negative samples. The identity of the PCR product from the unknowns was confirmed by comparing its specific melting temperature (T_m) with the T_m of the product from the positive control. Gel electrophoresis confirmed the authenticity of all amplicons; a single band was obtained for each fragment amplified. All LightCycler-based assays were performed in duplicate and showed identical results, indicating an high reproducibility of the assay. The LightCycler reduced the time taken to achieve results from PCR reactions and simplified the laboratory workflow by automation and by reducing the possibility of product contaminations. The results of the LightCycler-based analysis corresponded to the results found by conventional cycling (100% of agreement).
Conclusions: The LightCycler offers a standardized, fast, sensitive, and reproducible tool for in vitro amplification and sensitive-specific detection of the PCR products of SAP genes by melting curve analysis in *C. albicans*.

P958 Semi-quantitative detection of *Aspergillus fumigatus* in BAL fluids from patients with invasive pulmonary aspergillosis with a real-time PCR

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Objectives: To develop a quantitative or semiquantitative real-time PCR method for detection of *Aspergillus fumigatus* in BAL fluids and evaluate its value in the microbiologic diagnosis of invasive pulmonary aspergillosis (IPA), especially in differentiating between invasive disease and colonization of respiratory tract.
Methods: Amplification of aspergillus mitochondrial DNA was monitored by a pair of fluorescing probes in the LightCycler instrument. The analytical sensitivity of the assay was 100 *A. fumigatus* conidia per milliliter of BAL fluid (one conidium per reaction), and the assay was linear over four orders of magnitude above the detection limit. The reproducibility of the test was good, the coefficiency of variation (including DNA isolation, intra-assay and interassay variation) ranging between 1.0 and 2.7%. BAL fluids from 66 patients at risk of IPA and 33 immunocompetent controls were analyzed and the results were related to the clinical diagnosis established according to the recently published consensus criteria. Of the patients at risk, the diagnosis of IPA was proven in six (9%), probable in four (6%), and possible in five (8%).
Results: Amplification of aspergillus DNA was detected in 16/81 (20%) BAL fluid specimens from patients at risk and in 1/33 (3%) samples from immunocompetent controls. PCR was positive in 5/6, 2/4, and 4/5 of the patients with proven, probable and possible IPA, respectively, as well as in five patients in risk, but with no other evidence of IPA. Three patients with proven or probable IPA were positive in galactomannan antigen testing but negative in PCR, and two, positive in PCR but negative in antigen testing. With qualitative detection, the sensitivity of PCR per patient was 73%, specificity, 92%, and predictive values of positive (PPV) and negative (NPV) results, 73 and 95%, respectively. Using the threshold cycle <35 as a limit for

positive PCR, the specificity and PPV of PCR in the diagnosis of invasive aspergillosis in immunocompromised patients were 100%, but the sensitivity was only 45%, and NPV, 92%.
Conclusion: Semi-quantitative detection of *A. fumigatus* mtDNA in BAL fluid may be helpful in the diagnosis of IPA (in combination with galactomannan antigen), but the sensitivity of our assay was lower than expected on the basis of previous literature. The possibilities to improve the sensitivity are under evaluation.

P959 Identification of dematophyte species by 28*S* rDNA sequencing using a commercial kit

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Background: Dermatophytes are usually identified on the basis of macroscopic characters and microscopic examination of the cultures. However, identification of dermatophytes often remains difficult or uncertain because there are variations from one isolate to another.
Objective: To develop a method for rapid identification of the dermatophytes with high specificity and sensitivity on the basis of the DNA sequence of the genes encoding 28*S* rDNA.
Material and methods: DNA was extracted from fresh dermatophyte cultures on Sabouraud's agar medium. A part of the large ribosomal subunit RNA (28*S* rRNA) was sequenced after PCR amplification using the primers and the PCR mix included in the commercial MicroSeq D2 LSU rRNA Fungal Sequencing kit (Applied Biosystems).
Results: The selected sequence was unique and species-specific for all isolates of *Trichophyton rubrum*, *T. tonsurans*, *T. soudanense*, *Microsporum canis*, *M. audouinii*, *M. gypseum*, *Epidermophyton floccosum* and *T. mentagrophytes* var mentagrophytes. Two different sequence types were detected for *T. mentagrophytes* var. interdigitale. The differences between the species were generally due to single nucleotide polymorphisms.
Conclusions: The dermatophyte species could be easily, rapidly and routinely identified on the basis of a DNA sequence encoding a part of the 28*S* rRNA.

P960 Semi-nested PCR amplification of rDNA in the diagnosis of candidemia: comparison of amplicon detection by agarose gels with enzyme immunoassay

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Objective: To rapidly detect and identify four most commonly encountered *Candida* species viz. *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. glabrata* in clinical specimens by specific amplification of rDNA and to compare the sensitivity of amplicon detection by enzyme immunoassay with visual detection of amplified product in agarose gels.
Methods: The target for amplification was high copy rDNA. The universal outer primers amplified the 3′-end of 5.8*S* and 5′-end of 28*S* rDNA including the internally transcribed spacer 2 (ITS2) generating 317–423 bp fragments from the four commonly encountered *Candida* species. The species-specific primers derived from ITS2 and labeled with biotin, together with outer reverse primer labeled with digoxigenin amplified species-specific DNA in the re-amplification step of the *sn*PCR.
Results: The *sn*PCR employing both the detection methods was positive for genomic DNA recovered from 0.06 *Candida* cells in culture and 1 organism/mL in spiked serum specimens. Evaluation of *sn*PCR product detection by both the methods for specific identification of *Candida* species with 26 clinical *Candida* isolates showed 100% concordant results with Vitek and ID32C yeast identification systems. Further evaluation of *sn*PCR amplicon detection by both the methods for detection of *Candida* species in sera from culture proven ($n = 6$), suspected ($n = 10$) and healthy subjects ($n = 10$) showed concordant results.
Conclusions: The comparable sensitivity of amplicon detection by enzyme immunoassay and agarose gel electrophoresis implies that that the re-amplification step introduced before the amplicon is detected has resulted in maximum sensitivity of the protocol and that the incorporation of a more sensitive method of detection did not improve the sensitivity further. The agarose gel detection of the amplicons also reveals the size of the amplified product, is cheaper and more suitable for most of the laboratories in the developing countries for the detection of infecting *Candida* species in clinical specimens.
Supported by Research Administration projects grants MI 118, Kuwait University.

P961 A multiplex PCR using universal and species-specific primers to rapidly identify eight yeast species in positive blood cultures

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Objectives: The aim of this study was to evaluate a multiplex PCR method in a single reaction to directly identify eight yeast species in positive blood cultures. Three species are *Candida glabrata*, *Cryptococcus neoformans*, *C. krusei*, *C. albicans*, *C. guilliermondii*, *C. tropicalis*, *C. parapsilosis*, and *C. lusitaniae.*
Methods: The principle for designing primers for multiplex PCR was to select a consensus region within each species and to generate amplicons with difference sizes that can be separated by electrophoresis. Eight forward species-specific primers – CL, CP, CT, CGU, CA, CK, CN, and CGL – were designed based on sequences of the internal transcribed spacer (ITS) regions 1 and 2 determined in this study and on comparison of the sequences in the GenBank database to specifically amplify *C. glabrata*, *C. neoformans*, *C. krusei*, *C. albicans*, *C. guilliermondii*, *C. tropicalis*, *C. parapsilosis*, and *C. lusitaniae*, respectively. The ITS4 was used as a universal reverse primer. PCR amplification conditions were 3 min of denaturation at 94 °C, followed by 30 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min. To determine the lengths of PCR products, the amplicons were sequenced with an ABI Prism 377 automated system (Applied Biosystems). A total of 234 positive blood cultures containing yeasts were analyzed in this study. The blood yeasts isolated on subculture plates were identified by the conventional procedures based on phenotypic and biochemical reactions.
Results: The amplicon sizes were as follows: *C. glabrata* (631 632 bp), *C. neoformans* (516 bp), *C. krusei* (475 bp), *C. albicans* (402 bp), *C. guilliermondii* (185 bp), *C. tropicalis* (149 bp), *C. parapsilosis* (126 bp), and *C. lusitaniae* (116 bp). The method was used to analyze 234 positive blood cultures (237 isolates) from which the following species (strain number) were isolated: *C. albicans* (121), *C. tropicalis* (50), *C. glabrata* (29), *C. parapsilosis* (20), *Cryptococcus neoformans* (6), *C. krusei* (4), *C. guilliermondii* (3), *C. lusitaniae* (1), and other minor species (3). All isolates of the eight target species were identified by the multiplex PCR, resulting a test sensitivity of 98.7% (234/237).
Conclusions: The present method is simple, rapid, and approximately 99% of yeasts in positive blood cultures could be identified within 6 h.

P962 Karyotyping of Turkish environmental *Cryptococcus neoformans* variety neoformans isolates by pulsed-field gel electrophoresis

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Objective: Importance of opportunistic fungal infections has increased gradually in recent years due to expansion of immunocompromised situations. *Cryptococcus neoformans* has emerged as an important pathogen due to its virulence factors especially in cases of Acquired Immunodeficiency Syndrome. The most important ecological source of *C. neoformans* var. neoformans which is one of the two varieties of *C. neoformans* species is pigeon droppings. There are approximately 10 studies about isolation of *C. neoformans* from pigeon droppings in Turkey, however, this project is the first study defining genotypic characteristics of the isolates. In this project which is supported by the Scientific and Technical Research Council of Turkey, genotyping of national and environmental *C. neoformans* var. neoformans strains is aimed.
Methods: A total of 28 environmental *C. neoformans* var. neoformans strains isolated from 634 pigeon droppings from 54 different provinces of Turkey between years of 1996 and 1997 were enrolled in this project. Pulsed Field Gel Electrophoresis technique was applied as genotyping method.
Results: It was determined that the 28 strains constituted 26 different PFGE patterns.

Conclusion: Typing methods are indispensable components of epidemiologic investigations. Phenotypic methods such as antibiotyping, sugar assimilation, nitrate reduction and urease production might be insufficient sometimes as it is observed for these isolates (data not shown). PFGE as one of the most popular genotyping method was well enough to show genomic differences among cryptoccoccal strains as stated in previous reports and this study for epidemiologic purposes. The determined great heterogeneity indicates that a certain clonal population was not dominated in Turkey.

Fungi: pathogenesis/mechanisms/immunology

P963 Identification of new antifungal targets by comparative genomics and proteomics

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We used a comparative genomic approach to identify new antifungal targets. We first screened the *Saccharomyces cerevisiae* genome for small open reading frames (named smORFs) that have been overlooked in the previous annotation of the yeast genome. These smORFs were then searched against a comprehensive fungal protein sequence database to identify those that are conserved in other fungi. We further confirmed the existence of a subset of these small genes in *S. cerevisiae* by showing that they are expressed and are transcribed from the expected DNA strand using RT-PCR. From the list of expressed smORFs, we focused on those with homologs in both *Candida* and *Aspergillus* species and further tested these smORFs for essentiality. One of these genes, smORF 57, was essential in both *S. cerevisiae* and *C. albicans*. We searched for proteins interacting with smORF57 using the yeast two-hybrid system and identified three binding partners that are known to be part of the fungal mitotic apparatus. We additionally showed that the *Candida* gene CasmORF57 interacts with two of the respective *C. albicans* homologs. All three genes were found to be essential. The impact of this study on the identification of new essential, broad-spectrum, fungal specific targets is considerable. These results demonstrate that the smORF population provides an additional pool of potential therapeutic targets, both directly and through interactions with partner proteins.

P964 Expression of coagulase and hemolytic activity among *Candida* species

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Introduction: *Candida* species have the ability to produce enzymes that are known to mediate pathogenesis, particularly by facilitating hyphal invasion. Coagulase is an enzyme produced by different microorganisms that causes clot formation when in contact with plasma. Hemolysins are another example of microbial enzymes conferring pathogenic potential.
Objective: To evaluate the production of coagulase and the expression hemolytic activity by clinical strains of *Candida*.
Material and methods: A total of 144 *Candida* strains representing six different species (*C. albicans*: 70; *C. tropicalis*: 12; *C. glabrata*: 25; *C. parapsilosis*: 23; *C. krusei*: 11, *C. guilliermondii*: 3) isolated from clinical samples were selected. Two type strains from the American Type Culture Collection (ATCC) (*C. albicans* 90028 and *C. parapsilosis* 22019) were also included in the study. Tube coagulase test was prepared and analyzed at 4 h and whenever negative observed again following 24 h of incubation. The Pasteurex latex test is considered a specific test for the detection of *S. aureus* coagulase. We decided to evaluate its specificity by assaying the agglutination of its reagent with the same *Candida* strains (*S. aureus* ATCC 25923 and *S. epidermidis* ATCC 14990 were used as controls). Hemolysin production was evaluated using the method described by Luo et al. (1).

Table 1

	Tube coagulase test	Pasteurex latex test	Hemolysis
C. albicans	88.5%	68.9%	0
C. tropicalis	66.7%	33.3	0
C. glabrata	20%	0	0
C. parapsilosis	41.7%	8.3%	0
C. krusei	0	0	0
C. guilliermondii	33.3%	0	0

Results: The results regarding production of coagulase in the test tube, the agglutination of Pasturex reagent and production of hemolysis are detailed in Table 1.
Conclusion: Several species of *Candida* can produce coagulase, a known mechanism of pathogenicity. Many of them react unspecifically with the reagent to detect *S. aureus* coagulase, particularly *C. albicans*, as well as strains of *C. tropicalis* and a few of *C. parapsilosis*, probably due to similitude of antigens. Such a fact may cause mistakes in clinical laboratories. *C. krusei* does not produce coagulase. Despite the conflicting results of Luo et al. none of the tested strains was able to produce hemolise, independently from the site of isolation.

Reference:

1. Luo G., Samaranayake L.P., Yau J.Y.Y. Candida species exhibit differential in vitro hemolytic activities. *J Clin Microbiol* 2001; 39: 2971–4.

P965 Immunoblotting analysis of sera from patients with acute and chronic candidal and noncandidal vaginitis for IgE and IgG antibodies to *Candida albicans*

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Objectives: The purpose of this study was determination of IgE and IgG antibodies to *Candida albicans* in sera from patients with acute and chronic candidal and noncandidal vaginitis.
Methods: In this study, *Candida albicans* (*C. albicans*) was grown on medium. Then yeast cells were carefully harvested from the surface of the solid medium. After disruption of yeast cells, samples were centrifuged (25,000 rpm). Supernatants were collected and were used as a crude extract. Protein components in crude extracts of *C. albicans* were separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose filter. The components were detected by sera from patients with acute and chronic candidal and noncandidal vaginitis ($n = 80$, 20 patients from each of groups) and visualized by enzyme-labeled antihuman IgE and IgG antibodies in chromogenic substrate.
Results: In SDS–PAGE a total of 15 different protein bands (13–75 kDa) was observed in crude extract of *C. albicans* and in immunoblotting none of protein bands gave response with IgE antibodies. On the contrary, protein bands with molecular weight of 25, 47 and 52 kDa gave strong response with IgG antibodies in 100% of sera from chronic candidal vaginitis patients. Whereas, chronic noncandidal vaginitis patients were not reacted with any protein bands. Hundred percent of sera from acute candidal vaginitis patients gave strong response only with 47 kDa protein bands and 63.8% of chronic noncandidal vaginitis patients gave weak response with 47 kDa protein bands.
Conclusion: Our results showed that anti-*Candida* IgG to 25 and 52 kDa protein bands was strongly related with chronic course of candidal vaginitis.

P966 Antifungal activity of oregano oils (*Lippia graveolens* and *Origanum virens*) on dermatophyte species

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Dermatomycoses caused by dermatophytes are common superficial infections. The increasing resistance to antifungal compounds and the reduced number of available drugs urged the search for therapeutic alternatives. Previous research of our team demonstrated the antifungal activity of some thymus essential oils against dermatophyte species (1).
Objectives: Evaluate the antifungal activity of the essential oils of *Lippia graveolens* and *Origanum virens* (oregano oils) used in folk medicine in order to support their use as antifungal agents.

Material and methods: The composition of the oils was investigated by gas-chromatography and gas-chromatography/mass spectroscopy (2). The Minimal Inhibitory Concentration (MIC), determined according to the NCCLS protocol M 38-P, and the Minimal Lethal Concentration (MLC) were used to evaluate the antifungal activity against dermatophyte clinical strains (*Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. mentagrophytes* and *Epidermophyton floccosum*). The antifungal activity of their major components (carvacrol, gama-terpinene, *p*-cymene and thymol) was also evaluated.
Results: Both oils showed similar antifungal activity for all tested strains. MIC and MLC values are similar, ranging from 0.08 to 0.16 µm/mL. This is probably due to their chemical composition, mainly composed (over 70%) by carvacrol/gama-terpinene (*O. virens*) and carvacrol/*p*-cymene/thymol (*L. graveolens*).
Conclusions: This study showed the antifungal activity of these essential oils on dermatophyte species, supporting future therapeutical trials on dermatophytoses.
Acknowledgements: FCT, POCTI and FEDER (POCTI/40167/ESP/2001) for financial support; Prof. Dr. Armando Cáceres (CYTED-RIPROFITO) for providing *Lippia graveolens*.

References:

1. Pinto E., *et al*. In: Proceedings of the 33rd International Symposium on Essential Oils, 2002, p. 120.
2. Cavaleiro C., *et al*. *Biochem Syst Ecol* 2001; 29: 1175–83.

P967 The effects of UVB-irradiation on dermatophyte fungi

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The effects of UVB-irradiation on the various microorganisms have been investigated previously. However, little knowledge is available in the case of dermatophyte fungi. In this study, we tried to find out the morphologic and genetic changes following the UVB-irradiation on these fungi and analyze the obtained results by molecular methods. The following species of dermatophytes (*Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum canis*) have been isolated from patients and selected for investigation in this project. Equal amounts of dermatophyte species were cultured on Petri dishes contained Sabouraud Dextrose Broth (SDB), Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Agar with cyclohexamide and chloramphenicol (Scc). Also some slidecultures were prepared from these species. The cultured plates and slidecultures were then irradiated by UVB-light at the wavelength of 302 nm. The irradiated plates were kept in a dark room at room temperature. The effects of the irradiation on colonies have been studied based on macroscopical and microscopical methods in all species. For determining the possible molecular changes in dermatophytes due to UVB-irradiation, we next isolated high molecular weight DNA from non-irradiated as well as irradiated *T. rubrum* and compared them by gel-electrophoresis. The result of study indicated the inhibition of growth in irradiated colonies cultured all media. The longer irradiation time caused slower rates of growth in these fungi. Besides, the changes in shape of colonies and pigmentation was observed in some cases. The microscopic studies of irradiated colonies indicate deformation of hyphae and changes in size and shape of macroconidia in all species. Besides, changes in number of microconidia in some species were determined. The investigation of isolated DNAs from *T. rubrum* showed no differences in DNA banding patterns. These results indicate the susceptibility of dermatophyte fungi to UVB-irradiation. As we did not find any DNA changes due to irradiation, it can be concluded that the observed changes in growth-rate and deformation of mycelial and conidial bodies, may occur due to protein miss-folding or other changes in cell metabolisms.

P968 Propofol promotes *Aspergillus fumigatus* hyphal growth

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Aspergillus is a growing agent of disseminated fungal infections, particularly among neutropenic and transplant patients admitted in ICU units. Mycelium formation plays a crucial role in virulence, namely by promoting adherence to host cells and tissue invasion. Propofol, administered in a lipid emulsion, is a sedative drug widely used in critical care patients.

Objectives: To evaluate the promotional effect of propofol upon hyphal extension by *Aspergillus*.
Materials and methods: Five day old spores of clinical isolates of *A. fumigatus* (five strains) and *A. flavus* (four strains) were incubated with serial dilutions of propofol in RPMI 1640 medium (Sigma), at 37 °C. Samples (200 cells) were hourly checked for germination and hyphal extension (elongation), up to 24 h.
Results: Propofol-induced germination and hyphal formation by *Aspergillus*, noticeable soon after 5–6 h and particularly with *A. fumigatus*. The extending hypha usually shows embedding in a lipid layer, particularly at the hyphal tip.
Conclusions: Propofol is potentially associated to a promoted risk of nosocomial fungemia due its high potential of contamination, even when infused shortly after its use. The lipid layer around the hypha may, apart from promoting growth, prevent the antifungal activity of drugs and/or host defense mechanisms. Therefore, propofol seriously enhances the invasiveness and pathogenesis by *Aspergillus* organisms, promoting deep-seated fungal infections.

P969 Inhibitory effect of local anesthetics on germination and mycelium formation by *Aspergillus*

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Germination of spores and hypha formation is a crucial step in the pathogenesis of infection by *Aspergillus*. Local anesthetics possess a potent antifungal activity, namely upon *Candida* spp. (1, 2). It would be of interest to assess such effect on other emerging fungal pathogens.
Objectives: To evaluate the effect of lidocaine and bupivacaine on germination and mycelium formation of pathogenic species of *Aspergillus*.
Materials and methods: Five days old spores of 12 clinical isolates of *A. fumigatus* (six strains), *A. flavus* (three strains) and *A. niger* (three strains) were assayed for germination and mycelium formation in the presence of serial concentrations of lidocaine (0.06–0.25%) and bupivacaine hydrochloride (0.03–0.125%) (pure salts, free of preservatives, Sigma, USA), in RPMI 1640 medium (Sigma) at 37 °C. Germination and hyphal extension was hourly assessed by observation of 200 spores in a Neubauer's chamber, up to 48 h, in triplicate. Controls were prepared in plain RPMI medium.
Results: A significant, dose-dependent, pH-independent, inhibition of germination and hypha formation was observed in presence of lidocaine and bupivacaine, soon after 6 h of incubation, in all tested strains of *A. fumigatus*, *A. flavus* and *A. niger*. Germination of *A. flavus* and *A. niger* spores was completely abolished with 0.125% lidocaine. In the case of *A. fumigatus*, 0.25% lidocaine completely prevented mycelium formation by *A. fumigatus*, an effect that lasted up to 48 h. Bupivacaine reproduced this inhibitory effect but much lower concentrations than lidocaine.
Conclusions: Lidocaine and bupivacaine produced a potent inhibition of germination and hypha formation by *Aspergillus* species. Therefore, this antifungal activity should be better characterized in order to evaluate its role in future preventive or therapeutic attitudes to be taken in patients at risk of developing *Aspergillus* infections.

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P970 Inhibition of germination and mycelium formation by *Aspergillus* by local anesthetics: an effect related to ionic channel blockade

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Formation of mycelium following the germination of spores has been assumed an important virulence factor among fungal pathogens.
Local anesthetics (LAs), e.g. lidocaine and bupivacaine, are known to exhibit a potent antifungal activity, namely upon pathogenic species of *Aspergillus*.
Objectives: To determine if ionic channel blockade by means of selective calcium blockers could mimic the inhibitory effect of LAs on germination and hyphal extension by *Aspergillus*.

Materials and methods: Five days old spores of clinical isolates of *A. fumigatus* (three strains), *A. flavus* (two strains) and *A. niger* (two strains) were assayed for germination and mycelium formation in the presence of serial concentrations of selective calcium blockers, i.e. verapamil (0.5–1.5 mg/mL) and nifedipine (1–6 mg/mL) (Sigma), and a general blocker of ionic channels, i.e. lanthanum (0.25–2 mg/mL) (Sigma), in RPMI 1640 medium (Sigma), at 37 °C. Germination and hyphal extension was hourly assessed by observation of 200 spores in a Neubauer's chamber, up to 48 h, in triplicate. Controls were prepared in plain RPMI medium.
Results: A dose-dependent, pH-independent, inhibitory effect of germination and hyphal formation was obtained with the different blockers assayed, particularly with the selective blockers of calcium channels.
Conclusions: We conclude by the dependence of the inhibitory effect of LAs of blockade of ionic channel, particularly calcium channels. Our results stress the importance of calcium ion on morphology and pathogenesis of *Aspergillus*.

P971 Microscopic pathology of the kidney in rats fed *Fusarium graminearum*-inoculated diet

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Objective: Recently, *Fusarium* species have emerged as opportunistic pathogens in the immunocompromised human host. These fungi are frequently isolated from cereal grains, and produce some mycotoxins causing toxic disorders in mammals. In this study, we aimed to examine possible renal pathology in rats fed *Fusarium graminearum*-inoculated diet.
Methods: Twelve young adult Sprague–Dawley rats were randomly allocated into two groups: six animals fed *F. graminearum*-inoculated rice medium for 14 days (treatment group), and six animals fed noninoculated diet (control group). At the end of the 14-day interval, all rats were applied the technetium (99mTc)-labeled immunoglobulin G (IgG) scintigraphic imaging method for detection of foci of infection and inflammation, and then sacrificed. Subsequently, their kidneys were removed, and fixed in 10% neutral buffered formalin, and embedded in paraffin for light microscopic examination. Sections were stained with H-E.
Results: Increased uptake of 99mTc-labeled IgG was observed in the kidneys of treatment group rats. By light microscopy, the renal changes in the treatment group were characterized by the following findings: patch-like mononuclear cell infiltrations in cortex; many foci of necrosis and cellular debris; fibrosis in the necrotic areas; prominent atrophy of distal tubules; pleomorphism, karyoreksis, cytoplasmic vacuolation, apoptosis, and mitotic figures in the epithelial cells of proximal tubules.
Conclusions: It was concluded that dietary *F. graminearum* caused a chronic inflammation and great tubular damage to the rat kidney.

P972 Origins of relapses and resistance in recurrent vaginal candidosis

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Background: The cause of frequent relapses in chronic vulvovaginal candidosis (CVC) is still unknown. Microbiologic resistance is considered some times, but no proof still exists. Immunologic abnormalities may play a role in such patients.
Objectives: To study the possible microbial resistance and immunologic features in CVC with frequent relapses and treatment failures.
Methods: Study group included 53 female patients with CVC. Species identification was conducted using BBL Mycotube kit. Antifungal susceptibility testing was performed with ATB Fungus panel, complying with NCCLS standards. Immunological features and sensitization were examined with a set of specific methods.
Results: No variability was observed with causative species of *Candida*, regardless of disease duration and relapse frequency. *Candida albicans* was the only causative agent. No microbiologic resistance was observed for all azole antifungals. In this group, examination of immunologic status revealed significant decrease in CD4+ lymphocyte counts (CI with $P < 0.01$ vs. 94 donor controls) with non-significant decrease in IgA levels. Later we examined profiles of sensitization to standard *C. albicans* antigens in a set of five tests, allowing to reveal four types of hypersensitivity reactions (IgE, IgG-specific antibodies, bound IgG in neutrophil damage test, delayed hypersensitivity with lymphocyte IL-2 stimulation test) with control group of 22 healthy women. Specific IgE antibodies were found in 28/53 (52%) of CVC group and IgG neutrophil damage test (antibody-dependent cellular toxicity) was positive in 41 (77.3%), with significant difference from controls. Type III reactions (*Candida*-specific IgG) were significantly less frequent than in age-matching control group. Delayed type anti-*Candida* hypersensitivity was more frequent than in controls and more prominent after beginning of antifungal treatment.
Conclusions: A possibility of underlying immune deviation in CVC exists. This could better explain relapses and failures, rather than true microbial resistance that was not revealed in study group.

Antimicrobial susceptibility

P973 Antibiotic resistance of Gram-negative bacteria isolated from drinking water of South-western Greece

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Objectives: Determination of antibiotic resistance of Gram-negative bacteria isolated from drinking water samples.
Methods: Water samples were collected from 15 different regions of SW Greece. 105 water samples were analyzed for the presence of Gram-negative bacteria. Isolation of bacteria was performed by the filtration method. Identification of the bacteria was performed using API system method. The antibiotic resistance of all the isolates was assessed, applying antibiotic disk diffusion method. 13 antibiotics were used: Amoxicilline, Ampicilline, Cephalotine, Norfloxacine, Nalidixic acid, Chloramphenicol, Gentamycine, Amikacine, Neomycine, Nitrofurantoin, Tetracycline, Oxytetracycline, Sulfathiazole.
Results: Twelve different Gram-negative bacteria were isolated (*Aeromonas hydrophila*, *Citrobacter freundii*, *Enterobacter aerogenes*, *E. cloacae*, *E. sakazakii*, *Klebsiella oxytoca*, *K. pneumoniae*, *Morganella morganii*, *Salmonella arizonae*, *Serratia fonticola*, *Serratia marcescens*, *Escerichia coli*). 18.19% of *E. coli* was resistant to one or two antibiotics, 28.41% to three or four antibiotics, 3.41% to five or six antibiotics. *E. coli* showed 100% susceptibility to norfloxacine, chloramphenicol, gentamycine, amikacine. 80% of *A. hydrophila* were resistant to three antibiotics used. 75% of *C. freundii* isolated were resistant to three antibiotics and 25% were resistant to one antibiotic. *E. aerogenes* was resistant to four antibiotics whereas 50% of the *E. cloacae* were resistant to three antibiotics and 50% to four antibiotics. *E. sakazakii* was resistant to one antibiotic. *Klebsiella* species were resistant to two antibiotics. *S. arizonae* isolated showed susceptibility to all antibiotics used whereas *M. morganii* and *Serratia* species were resistant to four antibiotics.
Conclusions: Gram-negative bacteria isolates showed different antibiotic patterns. No statistical relationship was established between the antibiotic resistance pattern and the area of water sampling and the species of the bacteria. However, *E. coli* isolated from three distant regions showed different and characteristic antibiotic patterns. This is probably due to the different use of these antibiotics in each geographic area. The antibiotic resistance genes can naturally be transferred to other antibiotic sensitive bacteria via conjugation. These mechanisms of gene transfer aids in the horizontal transfer of antibiotic resistance and could pose a potential public health hazard by serving as a reservoir of antibiotic resistance genes.

P974 Bacterial isolates of intensive care and therapy units of a hospital in Kolkata and their meropenem sensitivity patterns

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Objectives: To find out common bacterial isolates in intensive care and therapy units and to observe sensitivity patterns of the newly introduced antimicrobial agent meropenem in our hospital in Kolkata.

Methods: The prevalence of different bacterial isolates was observed separately in different units within a span of 6 months from April to September 2002 and the prevalence rates of different bacterial isolates were compared. The sensitivity pattern of meropenem was determined according to NCCLS guidelines.
Results: Mainly three bacterial isolates were found highly prevalent in different units. These are *Klebsiella* sp. (K), *Pseudomonas* sp. (P) and *E. coli* (E). The prevalence rates in different units were quite different. In general ITU (K 15.0%, P 35.0%, E 25.0%) and in neurology ITU (K 22.2%, P 33.3%, E 30.6%), *Pseudomonas* sp. isolates were more while in neonatal ITU (K 58.8%, P 23.5%, E 14.7%) and in ICCU (K 35.7%, P 25.7%, E 18.7%) *Klebsiella* sp. Isolates were much more in number. The reason of increased *Klebsiella* sp. isolates may be due to the high prevalence of these organisms in environment of the City, which we observed earlier. Among all isolates 96.25% isolates were meropenem sensitive and the meropenem resistant isolates were mainly found in neonatal ITU.
Conclusions: *Klebsiella* sp., *Pseudomonas* sp. and *E. coli* were the main isolates of intensive care and therapy units of our hospital in Kolkata. Increased *Klebsiella* sp. isolates in these units may be due to high prevalence of these organisms in environment of the City. Meropenem sensitivity of the isolates was significantly high.

P975 In vitro antimicrobial sensitivity profile of the most common organisms isolated by a reference bacteriology laboratory in a prospective study, 1999–2002

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Objective: To evaluate the antimicrobial susceptibility pattern of the most common bacteria isolated at a reference Hospital.
Methods: A 4-year prospective survey was conducted to assess the microbiologic profile of all pathogens isolated from inpatients and outpatients referring to our center.
Results: From January 1999 to June 2002, over 100 different bacterial species were identified from clinical specimens, with *Staphylococcus aureus* (9961 strains, 3254 in the year 1999) and *Pseudomonas aeruginosa* (9670 isolates, 3202 in the year 2000), as the most prominent organisms, also borne by therapeutic problems. Throughout the 42-month study period, *S. aureus* strains remained completely sensitive to teicoplanin, vancomycin, rifampicin, chloramphenicol, and cotrimoxazole, while susceptibility of coamoxyclav ranged from 0 to 40.4% of tested strains, that of cefotaxime from 0 to 42.8%, clindamycin from 0 to 41.5%, erythromycin from 0 to 37%, gentamycin from 0 to 46.4%, and penicillin proved always ineffective; methicillin resistance ranged from 31.5% (year 2001) to 100% (year 2002). When analyzing the temporal trend, a significantly increased resistance was found for methicillin, coamoxyclav, cefotaxime, and gentamycin ($P < 0.001$, 2002 vs. 1999). *P. aeruginosa* proved sensitive over the entire 4-year period to ceftazidime, piperacillin-tazobactam, and imipenem, followed by tobramycin (74.8–100% of strains), amikacin (73.8–100%), aztreonam (70.2–100%), ciprofloxacin (68.5–100%), gentamycin (62.9–100%), ticarcillin-clavulanate (61.1–100%), piperacillin (68–72.6%), and mezlocillin (44–59.4%). A significantly increased resistance rate was found for gentamycin and ticarcillin-clavulanate ($P < 0.001$, 2002 vs. 1999).
Conclusion: A permanent survey plays a key role in the monitoring of epidemiology and resistance pattern of bacterial pathogens isolated in every geographic context. Empiric treatment and prophylaxis should be guided by local surveillance programs. *S. aureus* and *P. aeruginosa* were the most representative Gram-positive and Gram-negative bacterial pathogens in our 4-year prospective survey. In vitro assays conducted on nearly 10 000 isolates of each bacterial species showed an elevate effectiveness of glycopeptides, and also rifampicin, cotrimoxazole and chloramphenicol for *S. aureus*, and a fully reliable activity of ceftazidime, piperacillin-tazobactam and imipenem for *P. aeruginosa*, while the sensitivity of a broad spectrum of other tested antimicrobial agents was unpredictable, so that in vitro studies are strongly needed when such infections are of concern.

P976 In vitro antimicrobial susceptibility trends in clinically significant bacteria isolated in a general hospital during a 6-year period

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Objective: To investigate the resistance trends in certain antibiotics during a 6-year period in our Hospital, which does not have ICU.
Methods: From 1997 to 2002, 5,471 hospital and community-acquired bacterial stains were studied. The majority of the clinical specimens tested was urine (85.5%), and the rest (14.5%) included: pus, wounds, catheter tips, sputum, blood cultures, bile, prostatic secretions, etc. The identification of the isolates to the species level and the susceptibility testing to antimicrobials was performed by the API system (BioMerieux). The antibiotics included in our study were: ciprofloxacin (CIP), imipenem (IMP), nalidixic acid (NAL), ceftazidime (CAZ), gentamicin (GEN), oxacillin (OXA), vancomycin (VA) and teicoplanin (TEI).
Results: The majority of the isolates were obtained from urine specimens (69.2%). The predominant pathogen isolated was *E. coli* (44.7%), followed by *E. faecalis* (8.8%), *P. aeruginosa* (7.5%), *K. pneumonia* (6.3%), *P. mirabilis* (5.4%), *S. aureus* (3.7%) and other pathogens (23.6%). The resistance rates (%) of the most frequent pathogens for the years 1997–2002 for each of the antibiotics included in the study were as follows: *E. coli* 5.1–4.7–5.91–6.7–6.8–9.1 for CIP, 0 for all the years for IMP, 7.7–12.7–9.6–11.3–14.4–13.8 for NAL, and 2.3–3.2–3.6–4.6–4.83–6.1 for CAZ. *K. Pneumoniae* 8.5–8.3–6.8–7.8–9.6–10.6 for CIP, 0 for all the years for IMP, 29.7–27.9–21.3–19.3–13.5–16.3 for NAL and 10.6–11.6–12.2–8.3–10.7–13.2 for CAZ *P. aeruginosa* 34–38.5–29.1–29.5–41.3–47.1 for CIP, 6–12.9–9.3–11.6–12.3–19.1 for IMP and 6–17.9–16.6–17.9–16–19.1 for CAZ. *S. aureus* 22.2–25–31.5–33.3–21.4–48.2 for GEN and 22.2–25–44.7–30–27.8–53.5 for OXA. As for *E. faecalis*, we found 7.4 (5/67) VA resistant and 13 (9/67) TEI resistant strains in 1997, 1.4 (1/71) VA resistant strains in 2001 and 2 (1/50) VA resistant strains in 2002.
Conclusions: We observed an upward trend of the resistance rates of *E. coli* for CIP, NAL and CAZ. The *K. pneumoniae* strains showed a reduction of the resistance rates for NAL and *P. aeruginosa* strains showed an upward trend of the resistance rates for CIP, IMP and CAZ. The *S. aureus* strains also showed a significant increase of the resistance rates for GEN and OXA. We should notify that IMP kept a high effectiveness (100% sensitivity) for *E. coli* and *K. pneumoniae*.

P977 Activity of antibiotics against periodontopathogenic bacteria within epithelial cells

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Objectives: Periodontopathogenic bacteria are able to invade epithelial cells and to survive there. This might be a cause for chronicity of periodontitis. The purpose of this study was to determine the activity of different antibiotics against intracellular bacteria.
Methods: KB cells as a permanent epithelial cell line were infected by each of the following strains: *Actinobacillus actinomycetemcomitans* (A.a.) NCTC 9710, *Porphyromonas gingivalis* (P.g.) ATCC 33277 and JH16-1, and *Streptococcus constellatus* (S.c.) J012-2. P.g. JH16-1 and S.c. J012-2 were clinical isolates obtained from patients with severe recurrent periodontitis. After infection of the cell line extracellular bacteria were killed by addition of 10 µg penicillin/mL cell cultivation media. The effect of the antibiotics clindamycin, doxycyclin, metronidazole and moxifloxacin on the intracellular bacteria was tested after 2, 4 and 12 h. The concentrations used were the 1-, 10-, 50- and 100-fold MIC, which had been determined with planctonic, extracellular bacteria.
Results: The bacterial strains differed markedly in their ability to invade epithelial cells: 1.08×10^5 of intracellular P.g. ATCC 33277 were enumerated after 2 h, but only 0.5×10^3 up to 2×10^3 cfu were found for the other strains

at the same time. The 10-fold MIC of clindamycin reduced the number of S.c. after 4 and 12 h and the number of viable bacteria of the two P.g. strains were lower after addition of the 100-fold MIC. The 10-fold MIC of doxycyclin killed all A.a. after 4 h, whereas only little effects on the other strains were observed. Metronidazole showed only a reducing effect on the P.g. strains up to the 100-fold MIC. Moxifloxacin in the 10-fold MIC killed all A.a. The 50-fold MIC was able to eliminate all S.c. and the 100-fold MIC killed all intracellular P.g. ATCC 33277. The P.g. JH16-1 strain was not completely eliminated. The combination of metronidazole and moxifloxacin eliminated the ATCC strain by the 50-fold MIC and the JH16-1 strain by the 100-fold MIC for both antibiotics.
Conclusions: The results of this in vitro-study showed that moxifloxacin was the most active of the antibiotics against intracellular bacteria. But high MIC values were necessary for eliminating periodontopathogenic bacteria. Considering these results a possible application of this antibiotic in periodontitis patients should rather be a local than a systemic one and the efficacy has to be proven in clinical controlled trials.

P978 Interaction of aminoglycosides with the other antibiotic, combined in multiantibiotic drugs, analyzed by checkerboard and *E*-test methods

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Objective: Aminoglycosides with other antibiotics combined in some composed pharmaceutical preparations existing on Polish market. Combined antibiotic therapy has been used mainly to broaden the antibacterial spectrum and prevent the development of resistance. Checkerboard method is the most widely used technique to analyze antimicrobial interactions. The aim of this study was to compare above method with *E*-test assay for analysis of aminoglycosides (gentamicin, kanamycin, streptomycin and dihydrostreptomycin) interaction with other antibiotics (lincomycin, benzylpenicillin, amoxycillin, cephalexin, spectinomycin and erythromycin) combination on selected clinical bacterial strains.
Methods: Using checkerboard technique, serial dilutions of two antibiotics were prepared and combinations of six concentrations proportional to MIC value (from 1/4 × MIC to 2 × MIC) of both drugs were analyzed. Fractional Inhibitory Concentration (FIC) indexes were calculated from the checkerboard data. The strips of *E*-tests were placed on agar in a cross formation, with a 90° angle at the intersection between scales at their respective MICs. If only one antibiotic *E*-test was available, the second agent was added to agar. Strains: bacterial strains of: *Salmonella* sp., *S. enteritidis*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *E. cloacae*, MRSA and MSSA isolated from clinical sources were under investigation.
Results: Checkerboard results of all analyzed strains demonstrated synergism in 8% of combinations. However, additive effect was the predominant (65%). Neutral effect was obtained in 12.5% of combinations. In three cases (2 strains of *E. coli* and one *E. cloacae* isolate) in gentamicin combination with amoxicillin, inhibition effect was noticed. The checkerboard and *E*-test results corresponded in 55%. Twenty-one percentage of results obtained by both methods showed some discrepancies. These results concerned additive or neutral effects. Some investigators declare indexes, from over 0.5 up to 4, for neutral effects. Sharing this point of view, above 21% of discrepancy results can be counted as corresponded. In this situation, definite disagreement was observed only in 8% of obtained results.

P979 Antimicrobial susceptibility of bacteria causing complicated intra-abdominal infections

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Objective: To study antimicrobial susceptibility (AS) of the complicated intra-abdominal infections (CIAI) pathogens.
Methods: AS testing of the aerobic CIAI pathogens was performed by *E*-tests on Muller–Hinton II agar, of the anaerobes by broth microdilution. AS data were interpreted according to NCCLS (2001) recommendations and analyzed with WHONET software.
Results: 135 strains (55 aerobes and 80 anaerobes) isolated from 18 patients with CIAI in Smolensk were evaluated. Predominant bacteria were Gram-negative non-sporeforming anaerobes (NSA, $N = 50$) – *Bacteroides* spp. (37), *Prevotella* spp. (8), *Porphyromonas* spp. (3), *Dialister pneumosintes* (2); Enterobacteriaceae ($N = 35$) – *E. coli* (18), *K. pneumoniae* (6), *Proteus* spp. (5); *Clostridium* spp. ($N = 16$); less common bacteria were *Enterococcus* spp. (13); nonfermenters ($N = 9$): *P. aeruginosa* (5) and *Acinetobacter* spp. (3); *Eubacterium* spp. ($N = 5$); *Peptostreptococcus* spp. ($N = 4$). Most potent antimicrobials against Enterobacteriaceae were cefepime, imipenem and ertapenem (100% susceptible strains), ceftazidime and amikacin (97%), piperacillin/tazobactam, cefotaxime, ceftriaxone and ciprofloxacin (91%). High resistance was noted to ampicillin (71%) and gentamicin (43%). Most active against NSA were piperacillin/tazobactam, imipenem, ertapenem, metronidazole and chloramphenicol (98% susceptible strains), poor activity was noted for clindamycin (60% susceptible strains) and cefoxitine (68%). Similar susceptibility patterns were revealed in *Clostridium* spp. (imipenem, ertapenem and metronidazole – 100%, piperacillin/tazobactam and chloramphenicol – 88%, clindamycin – 62%, cefoxitine – 69%).
Conclusion: From the microbiologic point of view monotherapy with imipenem or ertapenem is appropriate for CIAI. Combination of piperacillin with tazobactam; cephalosporins III–IV or ciprofloxacin with metronidazole may be applied according to the in vitro AS data.

P980 Frequency of occurrence and antimicrobial susceptibility of bacterial pathogens from intensive care units in Latin American medical centers: report from the SENTRY Antimicrobial Surveillance Program

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Objectives: Resistance rates are usually higher in intensive care units (ICUs) due to a more focused antimicrobial selective pressure. This study assessed the antimicrobial susceptibility of bacterial isolates collected in ICUs from Latin American (LA) countries.
Methods: The isolates were collected in 10 medical centers (five countries) as part of the SENTRY Antimicrobial Surveillance Program. Beginning in January 2001, each participant center collected 50 consecutive and clinically relevant bacterial isolates from patients hospitalized in the ICU. The isolates were tested by reference broth microdilution methods (NCCLS) against a large number of antimicrobial agents.
Results: A total of 470 isolates were evaluated. The 10 most frequently isolated species were (*n*/%): *S. aureus* (SA – 102/21.7%), *P. aeruginosa* (PSA – 85/18.1%), *E. coli* (55/11.7%), *Klebsiella* spp. (43/9.1%), *Acinetobacter* spp. (ACB – 39/8.3%), *Enterobacter* spp. (38/8.1%), *Enterococcus* spp. (29/6.2%), coagulase-negative staphylococci (CoNS – 16/3.4%), *Serratia* spp. (16/3.4%), and *S. maltophilia* (13/2.8%). Resistance to oxacillin was detected in 54.9% of SA. Carbapenem resistance was detected in 42.4% of PSA and 28.2% of ACB. Polymyxin B (98.8% susceptibility [S]), piperacillin/tazobactam (64.7% S) and amikacin (63.5% S) were the most active compounds against PSA. Polymyxin B resistance (MIC > 4 mg/L) was also detected among ACB (97.4% S). ESBL-producing phenotypes occurred in 12.7% of *E. coli* and 54.5% of *Klebsiella* spp.; and 36.8% of *Enterobacter* spp. showed resistance to third-generation cephalosporins (AmpC hyperproduction). Only 82.8% of enterococci were vancomycin-S.
Conclusions: Resistance rates were extremely high among isolates from ICU patients in Latin American (LA) hospitals, especially among Gram-negative bacilli. The most alarming problems detected were imipenem and meropenem resistance among PSA and ACB, and ESBL mediated beta-lactam resistance and/or chromosomally based beta-lactamase (AmpC) among Enterobacteriaceae. Vancomycin resistance has clearly emerged among enterococci as a serious ICU problem in the LA region.

P981 Folic acid casei medium as a trimethoprim susceptibility test medium of *Lactobacillus paracasei*, *L. rhamnosus*, and *L. plantarum*

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Objectives: Documentation of probiotic bacteria often includes MICs. Lactobacilli have been reported to have intrinsic resistance to sulphonamides and trimethoprim. The ability of lactobacilli to use exogenous preformed folates and thymidine might explain the observed resistance. The susceptibility of lactobacilli to sulfadiazine and trimethoprim on different media

without thymidine was investigated in order to search for a phenotypic test method that could indicate the presence of resistance genes.
Methods: Etest susceptibility tests on MRS with thymidine phosphorylase, Defined Medium 1, and folic acid casei medium was compared. Strains included in the study were *Lactobacillus rhamnosus*, *L. paracasei*, *L. acidophilus*, and *L. plantarum*. *Enterococcus faecalis* ATCC 29212 was included as a trimethoprim sensitive control strain.
Results: *L. paracasei*, *L. rhamnosus*, and *L. plantarum* could be tested for trimethoprim resistance with Etests on folic acid casei medium. The MICs for the tested strains were 0.125–0.19 mg/mL for *L. paracasei*, 0.25–3 mg/mL for *L. rhamnosus* and 0.064–0.19 mg/mL for *L. plantarum*. *L. acidophilus* did not grow well on folic acid casei medium and could not be trimethoprim susceptibility tested. All lactobacilli had MICs of sulfadiazine >256 mg/mL on all media indicating intrinsic resistance.
Conclusion: The results show that *L. rhamnosus*, *L. paracasei*, and *L. plantarum* do not have intrinsic resistance to trimethoprim. However, an intrinsic phenotype could be observed on MRS medium. A total of 18 strains were susceptibility tested and the results indicate that none of the strains harbour a trimethoprim resistance gene. Trimethoprim susceptibility testing of some *Lactobacillus* species is possible with the use of folic acid casei medium.

P982 Comparative in vitro cytotoxic effects of Ornidazole, Metronidazole and Ciprofloxacin against *Trichomonas vaginalis* trophozoites

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Objective: To compare in vitro effects of Ornidazole, Metronidazole and Ciprofloxacin on Trichomonas vaginalis trophozoites in terms of minimal inhibition concentrations (MICs) and minimal lethal concentrations (MLCs).
Methods: A strain of *T. vaginalis*, isolated from a patient complaining vaginal discharge, was incubated in Trypticase-yeast extract-maltose (TYM) media at 37 °C. Then, *T. vaginalis* was cultivated in RPMI 1640 with 10% FCS under sterile conditions at 37 °C. To determine MICs and MLCs, drugs (Ornidazole, Metronidazole and Ciprofloxacin) at different concentrations were added into the culture plates. *T. vaginalis* trophozoites 25×10^4/mL in 1 mL of RPMI 1640 with 10% FCS were served as control. The numbers of *T. vaginalis* were counted by using Thoma counting chamber at 12, 24, 48 and 72 h.
Results: In this in vitro antitrichomonial experiment, at 12 h, although lesser effect was observed with 50 μg/mL, Ornidazole was effective at all doses, whereas Metronidazole was partially effective, and Ciprofloxacin was ineffective. MIC levels at 24 h for Ornidazole, Metronidazole and Ciprofloxacin were 50, 50, and 750 μg/mL. Corresponding MLC levels were the same.
Conclusions: In this in vitro study Ornidazole was found the most effective drug among three drugs tested against *T. vaginalis* in terms of MIC and MLC levels. It is interesting to note that Ciprofloxacin, although was not as effective as others, did have some cytotoxic effect on *T. vaginalis* trophozoites. Future in vivo studies may shed new light on this.

P983 Phenotypic antibiotic resistance of some enterobacterial strains adhered to an inert substratum. In vitro models for biofilm formation in liquid and solid media

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While planktonic bacteria causing acute disseminated bacterial infections can be controlled by conventional doses of antibiotics, adhered bacteria, responsible for chronic infections are resistant to antibiotherapy, due to their phenotypic antibioresistance, one of the most important consequence of the adhesion process. It was demonstrated that the currently used MICs are not active on bacterial biofilms. The purpose of this study was to determine the sensitivity levels of some opportunistic enterobacterial strains adhered to inert surfaces placed in 24-multiwell plates in liquid media and included in solid media, respectively, to subinhibitory concentrations, MICs, MBCs. In the experimental model in liquid medium the bacterial suspensions, preincubated with silicon-covered catheter sections (to promote the bacterial adherence and biofilm forming) were further incubated for 3 h in the presence of antibiotics and thereafter cultivated in fresh medium. The bacterial culture density, considered as indicator for the adherence capacity to the inert substratum and for bacterial recalcitrance to the usual doses of antibiotics, was assayed by spectrophotometer. The most interesting findings concern the chloramphenicol which was not active on adherent bacteria even in MBC, despite their sensitivity to chloramphenicol in planktonic phase. In the second model, we imagined an in vitro model for the biofilm polysaccharide matrix, represented by agar medium distributed in Petri plates in which the bacterial cells were embedded. The plates were further flooded with MICs, SICs, BMCs antibiotic solutions and the bacterial grow inhibition zones compared with those revealed by the classical disc diffusion method. Our study demonstrated that the bacterial strains sensitive to antibiotics in planktonic phase became resistant to the same doses of antibiotics when preincubated in the presence of the inert substratum, due to the biofilm formation that protects bacteria from the antibiotics action, by blocking the antibiotics diffusion. The bacterial cells included in agar medium exhibited decreased sensitivity to antibiotics. Phenotypic resistance exhibited by bacterial cells adherent to a substratum or included in biofilms amplifies the better known and recognized genotypic resistance phenomenon. These experimental models could be very useful for the appropriate testing of susceptibility and for an efficient treatment of medical devices associated infections.

P984 Bacterial conjunctivitis in primary care: causing agents and their susceptibility

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Objectives: Bacterial conjunctivitis is often diagnosed and treated by the general practitioner. In the Netherlands it is not common practice to confirm the diagnosis by culture, and therefore the infection is empirically treated by application of a topical antimicrobial agent. In the literature, data of the causing agents and their susceptibility to antibiotics are scarce. The aim of the study was to identify the bacterial cause and their susceptibility to antibiotics of conjunctivitis in general practice.
Methods: In 176 patients from 10 general practices with clinically suspected bacterial conjunctivitis using strict criteria, cultures were taken from the conjunctiva of both eyes by the general practitioner. Cultures were send to the laboratory for microbiology and performed according to the American Society for Microbiology. MICs from several topical antibiotics were determined using Etest. Critical MICs were used according to Dutch National Standards.
Results: Of the most affected eye, 58/176 cultures were positive with pathogenic bacteria, including *Streptococcus pneumoniae* ($n = 26$), *Staphylococcus aureus* ($n = 11$), and *Haemophilus influenzae* ($n = 11$). Of *S. pneumoniae*, four isolates were resistant against fusidin acid (MIC 3–16 mg/L), 4 against trimethoprim (MIC 8–32 mg/L), three against gentamicin (MIC 6–8 mg/L), and one against chloramphenicol (MIC 12 mg/L). Of *H. influenzae*, four isolates were resistant against fusidin acid (MIC 3–48 mg/L), two against trimethoprim (MIC 6–256 mg/L), and one against gentamicin (MIC 8 mg/L). Of *S. aureus*, six isolates were resistant against fusidin acid (MIC 12–256 mg/L), three against trimethoprim (MIC 8–32 mg/L), and one against gentamicin (MIC 8 mg/L). None of the isolates tested were resistant to Ciprofloxacin.
Conclusions:
1. The sensitivity of strict clinical criteria for bacterial conjunctivitis in general practice is low, when the culture is used as the golden standard.
2. From a bacteriologic point of view, only one-quarter of cases of clinically suspected bacterial conjunctivitis can be empirically treated effectively using local application of antibiotics.
3. In case of culture confirmed bacterial conjunctivitis, resistance against common topical antibiotics is relatively high.
4. General practitioners should be withholding in prescribing antibiotics for the empirical treatment of clinically suspected bacterial conjunctivitis.

Antibiotic policies and economics

P985 Comparison of length of stay between linezolid and vancomycin for the treatment of complicated skin and soft tissue infections from suspected or proven Methicillin-resistant *Staphylococcus* species: results from two randomized trials

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Objectives: In a randomized trial (Trial A), linezolid was shown to result in shorter duration of intravenous (IV) antibiotic treatment and length of stay (LOS) and higher proportion of first-week discharges among patients with complicated skin and soft tissue infections (CSSTI) from suspected/proven methicillin-resistant *Staphylococcus* species (MRSS) when compared with vancomycin (Pharmacotherapy 2001, 21: 263–74; IJTAHC 2002, 18: 540–54). A second trial (Trial B) showed a similar trend (2002 IDSA, 562). This study aims at confirming linezolid's effect by combining data from these trials.
Methods: Data were from two phase III trials (Trials A and B) that demonstrated equivalent efficacy between linezolid and vancomycin. The trials differed slightly in enrollment by geographic region but had same inclusion criteria and treatment regimens for CSSTI patients, who received up to 4 weeks of linezolid (IV or oral) or vancomycin (IV only), followed by up to 4 weeks of observation. The primary endpoint for the current analysis is LOS, which was estimated using Kaplan–Meier survival functions (KM). Results are presented as KM adjusted LOS means and medians, as well as proportions of patients discharged by the end of weeks 1–4. Arithmetic means for IV antibiotic treatment durations are also reported. Between-treatment differences in the KM functions of LOS, proportion of discharges and mean IV antibiotic treatment durations were tested with Wilcoxon test, Chi-square test, and Student's *t*-test, respectively.
Results: The combined sample showed that linezolid treatment was associated with a statistically significant shorter mean (1.7 days) and median (3 days) LOS, and approximately double the proportion of first-week discharges, possibly due to the linezolid group's shorter IV antibiotic treatment duration (see Table 1).

Table 1

				LOS (days, KM estimate)*			% patients discharged by the end of			
Study sample	Study antibiotic	*N*	IV days (mean)	Mean		Median (25–75 percentile)	Week 1	Week 2	Week 3	Week 4
Trial A	Linezolid	122	5.8	17.2		9 (5-26)	36	54	62	75
	Vancomycin	108	12.6	19.4		14 (8-24)	17	46	65	76
	P-value	–	<0.0001		0.052		0.001	0.24	0.69	0.93
Trial B	Linezolid	163	6.0	16.4		11 (5-21)	29	56	71	80
	Vancomycin	161	11.8	18.1		13 (7-22)	17	49	69	76
	P-value	–	<0.0001		0.10		0.015	0.22	0.75	0.39
Both trials combined	Linezolid	285	5.9	26.9		11 (5-24)	32	55	67	78
	Vancomycin	269	12.1	18.6		14 (7-23)	17	48	67	76
	P-value	–	<0.0001		0.01		<0.001	0.09	0.95	0.57

*The *P*-values for LOS are based on tests of the differences between the Kaplan–Meier survival functions for the two treatments from which the mean and median LOS are estimated.

Conclusion: Data from two prospective randomized trials confirm that linezolid use leads to shorter LOS, higher proportion of early hospital discharges, and reduced IV antibiotic treatment in CSSTI patients.

P986 Treatment with telithromycin leads to lower overall healthcare costs than clarithromycin in patients with community-acquired pneumonia: results from two independent, randomized, double-blind studies

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Objectives: To compare the clinical efficacy and economic impact of treatment with oral telithromycin (TEL) and clarithromycin (CLA) in adult outpatients with community-acquired pneumonia (CAP).
Methods: In two independent, randomized, double-blind, multicenter clinical studies, adult outpatients (Study 1, $n = 448$; Study 2, $n = 575$) with CAP received TEL 800 mg once daily for 10 days (Study 1) or 5/7 days (Study 2), or CLA 500 mg twice daily for 10 days (comparator regimen Study 1/2). Clinical and economic outcomes were followed over a 1-month period. The primary statistical hypothesis was equivalence of clinical efficacy in the per-protocol populations. Economic outcomes in the intent to treat populations were assessed by nonprotocol-driven CAP-related resource use. Unit costs were assigned as follows: additional antibiotics/medications using 1999/2000 (Study 1/2) Redbook prices; physician visits/tests/procedures based on Medicare's Resource Based Relative Value Scale; and hospitalizations using 1999/2000 (Study 1/2) American Hospital Association national average daily rates for short-term hospitalization.
Results: Equivalence in clinical efficacy of TEL and CLA was demonstrated in both studies – Study 1: 88.3% (143/162) vs. 88.5% (138/156) for 10-day TEL vs. CLA, respectively; Study 2: 5- and 7-day TEL, 89.3% (142/159) and 88.8% (143/161), respectively, vs. 10-day CLA, 91.8% (134/146). In Study 1, there were 16 CAP-related hospitalizations: 8 for TEL vs. 8 (with 2 ICU admissions) for CLA. In Study 2, there were 12 CAP-related hospitalizations: 4 and 1 (with 1 ICU admission) for 5- and 7-day TEL vs. 7 for 10-day CLA, respectively. TEL treatment resulted in a shorter total length of stay vs. CLA – Study 1: 42 vs. 64 days for 10-day TEL vs. CLA, respectively; Study 2: 47 and 14 days for 5- and 7-day TEL vs. 75 days for 10-day CLA, respectively. The mean per-patient cost of additional resource use for TEL was considerably less than that for CLA in both studies – Study 1: $382 vs. $666 for 10-day TEL and CLA, respectively; Study 2: $385 and $151 for 5- and 7-day TEL vs. $570 for 10-day CLA, respectively. Treatment with TEL resulted in mean per-patient savings of $284 vs. 10-day CLA (Study 1, 10-day), $185 vs. 10-day CLA (Study 2, 5-day), and $419 vs. 10-day CLA (Study 2, 7-day).
Conclusions: Two independent, randomized, double-blind studies confirm that TEL is an effective therapy for outpatients with CAP and is associated with lower overall healthcare costs than CLA.

P987 Total therapy cost for the preparation and administration of intravenous antibiotics: a single daily administration therapy vs. therapies requiring multiple daily administrations

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Objectives: To compare the costs of different antibiotics-based therapies having a comparable spectrum. To ascertain what cost-savings may be obtained by a therapy requiring a single daily i.v. administration as against therapies requiring multiple daily i.v. administrations.
Methods: The study was conducted in a large peripheral hospital in Belgium. The i.v. antibiotics were prepared centrally in the CIVA service dispensary. The entire process of preparation and administration of i.v. antibiotics was first analyzed. Each separate motion necessary for preparation and administration was timed. A total of 100 measurements were taken in the dispensary and on the wards. Additional data were then collected on staff salaries, the cost of materials and other costs making up the total cost of the therapy. An i.v. therapy with ertapenem 1 g, once daily (case 1), was compared with the following therapies with multiple daily i.v. administrations:
1. Piperacillin/tazobactam 4 g, four times daily (case 2).
2. Cefotaxime 2 g, three times daily in combination with metronidazole 500 mg RTU, three times daily (case 3).

The average cost per therapy was calculated for all three cases. Variable costs, fixed costs and overheads were included in the cost calculation. Variable costs are costs that increase as the number of preparations and administrations increases. They include a labor cost and a material cost. The fixed costs, by contrast, are independent of the number of preparations. In these fixed costs a distinction was made between a fixed labor cost, a fixed material cost, the investments and the maintenance of the investments. Finally, the overheads allocated to the clean room were also included in the calculation. The cost of the active products was disregarded.
Results: The time measurement results were as follows: the average preparation times for the three cases were, respectively, 5.62, 10.22 and 8.74 min. The average administration times for the three cases were, respectively, 1.38, 3.03 and 4.97 min. These working times were then converted into labor costs. Cost analysis produced the result set out in the table below.

Total cost	Case 1	Case 2	Case 3
Variable costs			
Labour cost, preparation, variable	2.723	4.639	4.021
Labour costs, administration, variable	0.578	1.268	2.076
Material cost	2.144	7.028	5.804
Fixed costs			
Labour cost, fixed	0.066	0.263	0.197
Material cost, fixed	0.087	0.348	0.261
Investments in material	0.121	0.484	0.363
Maintenance of material	0.118	0.471	0.353
Overhead costs	0.507	2.029	1.521
General total (Euro)	6.344	16.528	14.596

Conclusions: Our analysis reveals the main cost drivers to be the labor costs involved in preparation and administration and the cost of material. An i.v. antibiotic therapy requiring only one daily administration delivers a considerable cost-saving compared with therapies that require multiple daily administrations.

P988 Economic evaluation demonstrates that moxifloxacin IV/PO monotherapy is cost-effective to the UK National Health Service when compared with IV/PO amoxicillin/clavanulate ± clarithromycin in the treatment of community-acquired pneumonia

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Objectives: Community-acquired pneumonia (CAP) is associated with substantial morbidity, mortality, and financial cost. Improved treatments have potential to reduce these burdens on health care systems. A cost-effectiveness analysis was conducted from the UK National Health Service (NHS) perspective, comparing sequential IV/PO moxifloxacin (MXF) monotherapy to standard comparators in patients admitted to hospital with CAP.
Methods: Costs and consequences over 21 days were evaluated based on clinical cure rates 5–7 days post-treatment (test-of-cure (TOC) visit) and resource use reported for the TARGET multinational, randomized, open-label trial. Sequential IV/PO monotherapy with MXF 400 mg OD was compared with IV/PO amoxicillin/clavulanate (AMC) (1.2 g IV/625 mg PO TID) + clarithromycin (CLA) (500 mg BID) for 7–14 days in hospitalized patients with CAP. Since treatment effect on resource use (hospital days) was similar across countries, resource data (antimicrobial treatment, hospitalization, and out-of-hospital care) from all centers were pooled and valued using UK unit prices to estimate CAP-related cost per patient to the NHS.
Results: Clinical efficacy analyzes for ITT and PP populations, respectively, found 8.3% (95% CI: 3.1, 13.6) and 8.1% (95% CI: 2.9, 13.2) higher cure rates in the MXF group at TOC. Clinical outcomes were consistent with microbiological results in the trial. For economic evaluation, patients with indeterminate or missing outcome at TOC were conservatively assumed to be clinical failures, resulting in higher cure rates in the MXF group of 5.3% (95% CI −1.2%, 11.8%). Statistically significantly increased speed of response (first return to apyrexia 1 day sooner; $P = 0.008$) and reduction in hospital stay by 0.81 days (95% CI: −1.63, 0.01%) were observed in the MXF group. Treatment with MXF resulted in projected savings to the NHS of £230/patient, primarily due to shorter hospital stay. A cost-effectiveness acceptability curve demonstrated the probability of MXF being cost saving was 87%, and the probability of MXF being cost-effective relative to AMC ± CLA reached 92% at acceptability thresholds up to £1300 per additional patient cured.
Conclusions: MXF IV/PO monotherapy shows superior clinical outcomes and is cost-effective from a UK NHS perspective when compared with IV/PO AMC ± CLA in the treatment of CAP.

P989 Potential economic impact of an outpatient and home parenteral antibiotic therapy (OHPAT) programme for managing community-acquired skin and soft tissue infections (comSSTI's) in Scotland

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Objective: ComSSTIs (cellulitis and erysipelas) remain a significant cause of morbidity and hospitalization. The predominant pathogens remain MSSA and *S. pyogenes*. Intravenous therapy with high dose flucloxacillin (1 g qds) or oxacillin and benzylpenicillin, respectively, remains the established standard of care in most European hospitals. Administration of ambulatory IV therapy, usually with a single daily bolus injection of 1 g IV CEFTRIAXONE, remains an effective clinical and economic option. There is no evidence that this regimen in the ambulatory setting is associated with greater occurrence of resistance or adverse reactions. This study aimed to examine the economic impact of OHAPT in treating comSSTIs in Scotland.
Methods: Retrospectively review using the SMR1O inpatient discharge diagnosis data from Information Statistics Division Scotland (ISD) (http://www.show.scot.nhs.uk/isd) and the Dundee Infectious Diseases Units (DIDU) Outcomes Registry Database. The key diagnosis (ICD 10 codes) groups considered were cellulitis (LO30, 31, 32, 33, 38, 39) and erysipelas (A46X) over four consecutive years (1997–2001).
Results: Table.

	1997–1998	1998–1999	1999–2000	2000–2001
Total no. of annual impatient admissions in Scotland with above ICD 10 diagnosis	4434	4756	4997	5573 Total no. of episodes = 19,760
Mean duration of hospitalization (day)	5.33	5.69	4.7	4.91
Mean duration of hospitalization over four years (day)	5.16			
Mean cost per day of infection in Scottish ID unit (1999/2000)	GBP 279			
Estimated annual cost to Scottish health Service for all hospitalized comSSTI	1440 × 19,760 divided by 4 = GBP 7.1 million			

Conclusions: OHPAT was implemented in the (DIDU) in 1997/98. Data from the Dundee outcomes registry revealed a mean reduction in length of hospitalization from 6.1 days (1996/1997) to 2.9 in 1999/2000 – a reduction of 48%. If this is applied to Scotland then OHPAT would reduce hospital stay roughly by half with a resultant annual reduction in cost of about GBP 3.8 m. If IV therapy is to remain a standard of care for more severe comSSTIs then OHPAT should be considered as an important clinical and cost effective option. These data would be applicable to European practice.

P990 The (direct) cost-efficacy of managing severe malaria in children aged 6–59 months following the WHO guidelines in two district hospitals in Cameroon

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Since efforts to eradicate malaria failed in the 1970s, Sub-Saharan African countries have learned to live with malaria, which remains the main cause of morbidity and mortality in children less than 5 years old. There is increasing concern about the cost of health care, especially as health insurance policies

have been proliferating. Our main aim was to determine the direct cost and efficacy of case-management of severe malaria following the current WHO guidelines in children aged 6–59 months. From January 1 to August 31, 2000, 148 children (aged 6–59 months, and who presented with at least one feature of severe malaria) were recruited by consecutive sampling, at the Djoungolo and the Mfou District Hospitals. Treatment according to WHO guidelines was implemented and there was rigorous in-patient monitoring and the outpatient follow-up. There were 72 girls and 76 boys; the mean age was 23.1 ± 13.1 months and the commonest clinical forms of severe malaria were: generalized convulsions (54.7%), prostration (43.2%) and severe anemia (14.9%). Most children (95.9%) were completely cured, 2.0% died and there were no neurological deficits over 1 month follow-up. We estimate the cost of hospital management of each episode of severe malaria at 26,000–36,000F CFA and the overall direct costs (before and during hospitalization) at 27,000–39,000F CFA. We conclude that the current WHO guidelines are efficacious, but expensive as compared with the standard of living in Cameroon.

P991 Potential impact of IV-Oral switch on length of stay in Gram-positive infection

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Objectives: To prospectively evaluate the potential impact that switching from a glycopeptide to an equally effective oral agent to treat resistant Gram-positive infections would have on IV line use and length of stay (LOS) in a London Teaching Hospital Trust.
Methods: Patients on glycopeptides for 5 days or more were reviewed by a pharmacist + Medical Microbiologist or ID physician. Patient demographics, antibiotic prescribing indications, monitoring, comorbidities, laboratory investigations and clinical course were recorded. Standardized IV-oral switch (previously piloted and implemented) and discharge on oral therapy criteria were applied to each patient. The difference between actual and potential resource utilization was recorded. Statistical analysis was carried out using SPSS.
Results: Two hundred and eleven patients received glycopeptides for over 5 days, 183 (87%) vancomycin, 20 (13%) teicoplanin, and 8 (4%) both (at different times), 176 (83%) had a positive microbiology result of which 122 (69%) were MRSA, 60 (28%) patients were prescribed glycopeptides within 4 days of admission. Sixty-two (29%) patients could have had a reduced LOS potentially saving 649 bed days over 6 months. A further 15% could have benefited from reduced time on IV therapy. Reduced LOS was significantly associated with site of infection (skin and soft tissue), specialty (orthopedics or plastics), organism (MRSA) and number of other antibiotics (P less than 0.01–0.001, chi-square or logistic regression as appropriate). Importantly whether a patient could switch from IV-oral therapy and LOS could be reduced would have been successfully predicted in 76% cases in patients with skin/soft tissue infection, were managed in orthopedics or plastic surgery specialties, were on less than five antibiotics and did not have specific comorbidities.
Conclusions: There is significant potential to reduce IV line use and LOS in at least a quarter of patients with resistant Gram-positive infections by using an equally effective oral agent. This would have a considerable impact on bed use in some specialties. IV-oral switch programmes should be evaluated further.

P992 Association between inappropriate initial empiric antibiotic therapy and healthcare resource use among patients undergoing surgery for community-acquired intra-abdominal infections in Spain

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Objective: To assess the association between inappropriate initial empiric antibiotic therapy and healthcare resource use among patients undergoing surgery for community-acquired intra-abdominal infections (IAI) in Spain.
Methods: Records of patients who underwent surgery for community-acquired IAI from 10/1998 to 08/2002 in hospitals in Spain were reviewed. Initial empiric antibiotic therapy was classified as inappropriate if at least one pathogen was resistant to all antibiotics in initial regimen in case of positive culture, while in case of negative/missing culture, the Surgical Infection Society guideline (Bohnen 1992) was used for classifying appropriateness of initial empiric therapy. Healthcare resource use was measured as hospital length of stay in days (LOS) and days on IV antibiotic therapy. Semi-log least square regression analyses were performed to assess associations between inappropriate therapy and healthcare resource use measures, after adjusting for patients' demographic and comorbid characteristics and site/type of infection.
Results: Four hundred and twenty-five patients were included of whom 40.5% were female. Mean (SD) age of these patients was 53.2 (20.7) years. Of these patients, 387 (91%) received appropriate initial empiric therapy while the rest were on inappropriate therapy. Most common sites/type of IAI were appendicitis with peritonitis (31.5%) and appendicitis with peritoneal abscess (17.2%). Mean LOS (SD) was 12.4 (10.5) and 16.3 (14.0) days among patients with appropriate and inappropriate therapy, respectively ($P = 0.24$). Mean (SD) days on IV antibiotic therapy was 8.6 (6.9) for patients on appropriate initial therapy while 12.5 (10.1) days for patients on inappropriate therapy ($P = 0.02$). Multivariate analyses showed that after adjusting for type/site of infection and patients' characteristics, inappropriate therapy was associated with almost 18% increase in LOS ($P < 0.05$) and 28% in IV antibiotic days as compared with appropriate therapy ($P < 0.05$). Confining the analysis only to patients with positive culture ($n = 199$), showed that inappropriate therapy was associated with 57% increase in LOS and 35% increase in IV antibiotic days as compared with appropriate therapy after controlling for other factors.
Conclusion: Among patients undergoing surgery for community-acquired IAI, inappropriate initial empiric antibiotic therapy was associated with increased healthcare resource use through increase in length of hospital stay and length on IV antibiotic therapy.

P993 National programme for antibiotic policy: the Bulgarian experience

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Objectives: A national program for antibiotic policy (NPAP) was developed for both appropriate antibiotic treatment of patients and containment of antimicrobial resistance (AR). The aim of this work is to share our experience and some difficulties in implementation, which may be common with other Eastern European countries.
Methods: It was developed by an expert multidisciplinary committee and following wide discussions, was approved by the Ministry of Health in 2001. It included: (A) Quality Control (QC) in the Clinical Microbiology Laboratory (CML) and AR surveillance, (B) rational antibiotic treatment and prophylaxis, (C) control of infection (CI), (D) education. A reference CML was created to perform QC and AR surveillance. National consumption of antibiotics was registered by the Bulgarian Drug Agency. Hospital committees developed institutional guidelines on AP and CI. Medical education was enforced.
Results: NPs for Good Clinical Practice and CI are at early stage of preparation. Problems encountered were (1) over the counter sale of antibiotics is not controlled, (2) no good coordination between the institutions involved, (3) funding is not sufficient: sometimes patients have to buy more expensive antibiotics by themselves, (4) resources for CML are 10–20-fold less than in EC countries.
Conclusion: Reality is more complex than theory: to create a working NPAP, world-wide and European recommendations should be considered together with the particular socio-economic, political and healthcare status of the country involved. Awareness and responsibility of medical societies should be combined with coordinated actions of the institutions and funds from government, health Insurance and international authorities.

P994 Antimicrobial misuse in patients with bacteraemia

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Background: Studies have shown that antimicrobials are not always properly used in clinical practice. Misuse of such drugs may be harmful for patients and may produce unnecessary expenses. Research is needed to clarify the magnitude of the problem and to know its determinants.
Methods: The medical charts of all patients with bacteremia, detected from January to April 2001 in the Hospital General of Castellon, were reviewed by two Infectious Disease physicians. They assessed (1) if blood cultures represented true infection or contamination and (2) if antimicrobials were prescribed according with current recommendations [Med Lett 2001; 43 : 69; Sanford Guide 2002]; when both physicians disagreed, they jointly discussed the case and reached a consensus. The possible association of age, sex, department, ward, length of hospitalization, cultured microorganism, number

of predisposing factors of infection, true infection or contamination, source of bacteremia, and mortality, with appropriate or inappropriate prescription of antimicrobials was appraised with a logistic regression. The approximate cost of improperly employed drugs was estimated.
Results: A total of 190 patients were included. The median of age was 58 years; 116 (61%) were male. The agreement between both physicians about the clinical significance of blood cultures and about the appropriateness of antimicrobial prescriptions were good (Kappa 0.897 and 0.876, respectively, both $P = 0.000$); 68 cultures (36%) were classified as true infections; 28 patients (13%) received antimicrobials that were considered inappropriate. *Staphylococcus* spp. other than *S. aureus* represented 97 isolates (51%) and *E. coli* 19 (10%). An association was found between inappropriate antimicrobial prescription and: longer hospitalization ($P = 0.011$), true infection ($P = 0.011$) and known source of infection ($P = 0.001$). The estimated cost of unnecessarily employed antimicrobials was €11,000.
Conclusion: Nonadherence with recommendations on antimicrobial prescription was common and widespread among physicians of all departments, and provoked substantial unnecessary expenses.

Epidemiology of parasite infection

P995 An epidemiological study of cutaneous leishmaniasis in a central focus of the disease in Iran

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Objectives: In the past decade cutaneous leishmaniasis had a high outbreak in Yazd province, central Iran. The aim of this study was to determine the epidemiological situation of the disease for designing and suggestion a control program.
Methods: The study was carried out in 3 villages around the Ardakan county, Yazd province. Total population were visited and examined for scar(s) or sore(s). A questionnaire was completed for any family by asking them about age, sex, history of previous infection, etc. Smears were prepared from every case with acute lesion. The RAPD-PCR test was used to detection the parasite species.
Results: More than 3000 persons were visited and examined. From them 747 cases had acute lesion(s) and in 920 cases we found scar(s). The infection was observed in all age groups. The prevalence of the diseases was evaluated 24.7%. Results of the parasitology examination showed that *Leishmania major* is the agent of cutaneous leishmaniasis in this area.
Conclusion: The findings showed that an epidemic of zoonotic cutaneous leishmaniasis (ZCL) was happened in this focus. *L. major* was isolated for the first time in the area, in this study. Because of the epidemiological aspects of ZCL, we suggest the reservoir hosts (gerbils) be controled with toxic bait in a radius of 500 m around the villages.

P996 Occurrence of *Plasmodium* spp. among the suspected cases in Diyarbakir, Turkey

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Objectives: The aim of this study was to outline the total number of *Plasmodium* positive blood samples in Diyarbakir in the last 6 years according to the data of Malaria Eradication Chamber. Also, to evaluate the types of malaria seen in the region, the data of blood donors and to compare the situation of Diyarbakir which has priority for malaria, with the other parts of the country.
Methods: Blood samples of 751 312 people were evaluated for the presence of *Plasmodium* parasites between 1995 and 2000. These samples were collected for the aim of active or passive surveillance studies and check studies. Among the samples, 80,330 were found positive for *Plasmodium* spp. Thin and thick blood smears and direct microscopy technique were used to detect the parasites.
Results: Numbers of *Plasmodium* positive donors varied between 2581 and 26 912 during 1995–2000 period. Percentages of male patients varied between 52.6 and 58.0% and of female patients between 42.0 and 47.4%. Infection occurred most predominantly in 15–44 years of age. According to the monthly data of all examined years, malaria infection was seen intensively between may and September. *Plasmodium vivax* was identified as the causative agent in the positive blood smears.
Conclusions: Malaria infection is still important in Diyarbakir and districts. According to the inspected data in this study, 31.5–35.6% of the annual malaria cases of Turkey were seen in Diyarbakir between 1995–2000. Infection rates are higher in males than females and in 15–44 years of age group than the rest of the ages.

P997 Is *Strongyloides stercoralis* endemic in Greece?

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The aim of this study was: (a) the detection of an endemic area of *Strongyloides stercoralis* in our country (b) the investigation of existent forms of the parasite in the environment, the infectivity of the strains which encounter in the soil and the transmission frequency of the infection in the general population. The detection of ova and rhabditiform/L1 larvae in the urine sediment of a patient with nephrotic syndrome under immunosuppressive therapy, the repeatedly negative fecal examination for ova and parasites and the excellent clinical condition of the patient, created the suspicion of urine contamination from the soil. The culture of the urine sediment with Harada-Mori tube filter paper method showed all the stages of the indirect or heterogenic cycle (free living male and female worms, ova and rhabditiform/L1 larvae) except for the infective filariform/L3 larvae. Testing a large quantity of soil from and around the area where the urine selection pot was kept, with the Baerman method, revealed Rhabditiform/L1 larvae. Despite the detection of *S. stercoralis* endemic area in our country, the autochthonous human infection is extremely rare, since during the last 16 years among a large number of patients examined in our laboratory only one case of asymptomatic enteric strongyloidiasis was diagnosed. The fecal culture (using the same method) of two patients from Egypt with enteric strongyloidiasis, showed only infective filariform/L3 larvae (direct or homogenic cycle).
Conclusions: (a) This restricted epidemiological study provides evidence for the endemicity of *S. stercoralis* in Greece. (b) The autochthonous human infection in our country is rare, probably because of the existence of strains adapted to complete the indirect cycle in the environment without giving infective filariform/L3 larvae. On the contrary, strains of *S. stercoralis* Rhabditiform/L1 larvae detected in feces of patients with strongyloidiasis yielded only infective filariform/L3 larvae.

P998 Prevalence of microfilaria infection in a 12-month period in Spanish hospital

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Objective: To find out the frequency of imported microfilaries in travelers and immigrants coming from endemic areas.
Methods: All samples were collected in Hospital Carlos III from January to December 2002. The analysis was based on the microbiology laboratory data. Research for microfilaries in blood and skin were done in laboratory according to standard techniques. The identification were performed by an expertized parasitologist.
Results: A total of 1017 samples were analyzed. 663 were collected from blood and 354 from skin (169 from gluteus, 158 from scapula, and 27 from other localization). We found 44 positive samples in blood (6.64%), and 20 in skin (5.65%). In the positive blood samples, 31 were *Mansonella persans* (70.45%), 12 Loa-Loa (27.27%). One case had both *M. persans* and Loa-loa (2.27%). The patients came from Equatorial Guinea (30 cases of *M. persans* and 12 cases of Loa-loa) and Congo (1 case with *M. persans* and Loa-loa). All the positive skin samples were caused by *Oncocerca volvulus*, 12 were obtained from gluteus, and 8 from scapula. The patients came from Equatorial Guinea (18 cases) and Mali (2 cases).
Conclusions: In the last few years, we have observed in our hospital a growth in the number of cases of microfilaria infection due to the increasing arrival of

immigrants coming from endemic areas. We suggest clinicians should be aware of this kind of infections.

P999 Seasonal variation in bacterial pathogens isolated from stool samples in Karachi, Pakistan

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Objectives: Diarrheal diseases contribute significantly to morbidity and mortality in the developing countries. Although a few publications have been cited in literature from Pakistan on diarrheal diseases, none of the investigators have ventured into describing the seasonal variation of the commonly found bacterial pathogens in our setting. We wanted to describe the seasonal variation of various enteric pathogens found in an urban setting in Pakistan.
Methods: We planned a retrospective descriptive study of all the stool samples submitted from within Karachi to the Aga Khan University Hospital Laboratory over a period of 5 years (January 1997–December 2001) in order to determine the commonly isolated bacterial pathogens and to predict their seasonal variation.
Results: A total of 16,379 stool samples were included in this review. Bacterial isolates were found in 6670 stool samples (culture detection rate = 40.7%). The mean age at the time of culture of each subgroup was under 1 years group (6.58 ± 3.1 months), 1–5 years (2.13 ± 0.94 years), 5–14 years (8.3 ± 2.6 years) and adults (43.2 ± 18.5 years). Male:female ratio was 1.2:1. *Vibrio cholera* 01 Ogawa (32.8%), *Campylobacter jejuni* (17.3%), Enteropathogenic *Escherichia coli* (9.9%), *Salmonella paratyphi* b (6.6%) and *Shigella flexneri* (6.2%) were the most common organisms isolated. These organisms show a distinct seasonal variation with summer predilection (Fig. 1).

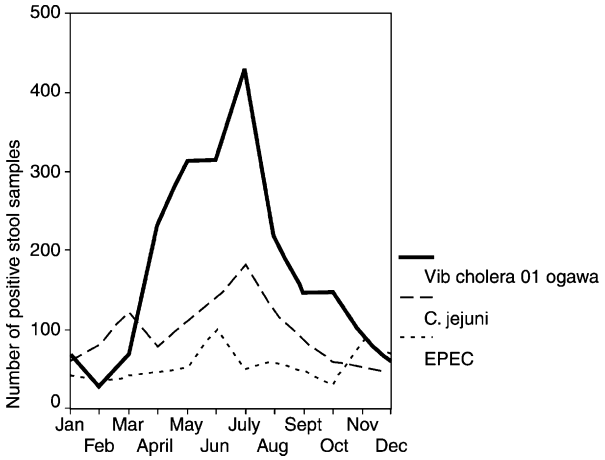

Figure 1 Seasonal variation of three most common bacterial isolates (1997).

Conclusions: In contrast to the previous studies from South Asia which have identified *E. coli*, followed by *V. cholerae* as the most common enteric isolates, we found *V. cholera* 01 Ogawa followed by *Campylobacter jejuni* as the most common enteric pathogens isolated in an urban setting. It is important to consider seasonal variation when empirically treating diarrheal diseases in our setting.

Parasitology: basic science

P1000 Characterization of a divalent metal ion transporter in adult schistosomes

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Objectives: Schistosomes are parasites of the portal veins of humans, where they derive nutrition by digesting host erythrocytes. The parasites have a high requirement for iron and store iron in two isoforms of ferritin, Fer1 and Fer2. Fer1 is highly expressed in females and is most likely associated with ferritin complexes observed in vitelline cells by electron microscopy. It would appear therefore that schistosomes have a strong requirement for iron in embryogenesis, but the mechanisms for iron regulation and transport in the parasite have been entirely unexplored. We are investigating key iron transporter proteins in schistosomes to determine whether the parasites have an iron regulatory system homologous to that of their human hosts. Here we describe studies on a schistosome homologue of a mammalian divalent ion transporter (DMT-1), which, in mammals is involved in uptake of iron from the gut by enterocytes.
Methods: We used nested PCR using degenerate primers based on consensus regions of DMT-1 genes to amplify schistosome sequences from adult *Schistosoma mansoni* cDNA. Amplified products were cloned into vectors and sequenced. Further characterization of the gene and product was carried out using Southern and Western blotting, as well as immunofluorescence microscopy using heterologous antisera raised against mouse DMT-1.
Results: We found that *S. mansoni* expresses a protein with significant homology to DMT-1 of mammals and other organisms. Western blot analysis showed the native protein to be approximately 110 kDa. The molecule was encoded by a single gene. Immunofluorescence microscopy suggested that the schistosome protein is expressed in the tegument and vitelline cells of females.
Conclusions: Schistosomes express a molecule with significant homology to mammalian DMT-1. The function of the schistosome protein has yet to be determined, but from its localization pattern, it is tempting to speculate that the molecule is involved in cellular uptake of iron. We are currently investigating whether other homologues of the mammalian iron regulatory molecules are also expressed in these parasites. Iron appears to play an important role in egg formation in schistosomes. In view of the importance of the eggs in the pathology of schistosome infections, a better understanding of the role of iron in embryogenesis and its regulation in adult worms may open new avenues for future control of schistosomes.

P1001 In vitro activities of telithromicin (HMR 3647) and primethamine on *Toxoplasma gondii* in cell culture

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Objectives: Toxoplasmosis is an infection disease caused by the obligate intracellular parasite *Toxoplasma gondii*, and receives upmost attention because of its potential to cause congenital infection. The infection is also severe and can be life threatening in immunodeficient or immunocompromised individuals. Drugs currently used for the treatment of toxoplasmosis are not always effective and/or may be deleterious for pregnant women and immunocompromised patients with acute toxoplasmosis, and for those with the ocular disease. The search for more effective and less-toxic drugs and therapeutic approaches have been continuing. The aim of this study was to investigate the therapeutic activities of telithromycin (HMR 3647) and pyrimethamine in vitro using HeLa cell cultures maintained intracellularly infected with *T. gondii* RH strain.
Methods: Monolayer HeLa cells in 96-well tissue culture plates were first infected with the tachyzoites obtained from peritoneal fluid of mice infected with *T. gondii*. The drugs were then added and their therapeutic activities were assessed after 24, 48 or 72 h postinfection by detecting the amount of *T. gondii* antigens in methanol fixed cells by using ELISA employing antitoxoplasma antibodies. The toxicities of drugs and the tachyzoites for HeLa cells were also determined by dye uptake assay.
Results: The significant inhibition of replication of the tachyzoites even at 48 h postinfection was obtained with telithromycin at 0.5 μg/mL ($P < 0.01$), and after 72 h of incubation even a concentration as low as 0.05 μg/mL ($P < 0.05$) was effective. The 50% inhibitory concentrations (IC50) of telithromycin and pyrimethamine at 72 h postinfection were estimated 0.81 and 0.16 μg/mL, respectively. None of the tested concentrations of both drugs demonstrated toxicity for HeLa cells even after 72 h of incubation.
Conclusions: Our results showed that telithromycin (HMR 3647) significantly inhibited intracellular replication of the tachyzoites of *T. gondii* RH

strain as did pyrimethamine, and that further studies with regard to use of telithromycin in combination with other drugs in animal models of toxoplasmosis may be of help for designing new treatment regimens for toxoplasmosis in humans.

P1002 Tissue cysts of the RH strain of *Toxoplasma gondii* induced by treatment of murine infection with atovaquone combined with pyrrolidine dithiocarbamate

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Objectives: During evaluation of the efficacy of atovaquone (ATO) combined with pyrrolidine dithiocarbamate (PDTC) in murine *Toxoplasma gondii* infection induced with tachyzoites of the mouse-virulent RH strain, brain cysts were observed in some survivors. Since this strain is considered to have lost its cyst-forming capacity, and conversion of RH tachyzoites into cysts in mice has previously been shown exclusively following treatment with sulphadiazine, this new protocol for in vivo development of RH strain cysts is described.
Methods: Groups of 12 Swiss Webster mice infected intraperitoneally with 102 tachyzoites each were treated with ATO at 5, 25 and 100 mg/kg/day alone and combined with PDTC at 250 mg/kg/day administered in food from day 1 post infection (p.i.) for 14 days. Six weeks p.i., the euthanized survivors were examined for residual infection. Virulence of the recovered cysts was examined by subinoculation into fresh mice.
Results: Whereas the survival of all treated mice was prolonged in a dose-dependent manner, 1, 2, and 4 mice treated with PDTC combined with 5, 25 and 100 mg/kg ATO/day (8, 17 and 33%), respectively, survived the 6-week observation period. However, 2 of these showed clinical toxoplasmic encephalitis only days before its end, and each was found to have harbored >3000 brain cysts. In addition, in brain homogenates of another 2 survivors without clinical signs, 107 and 267 cysts, respectively, were revealed, giving a total of 57% (4/7) cyst-harboring survivors. These findings were associated with highly positive *T. gondii* serology. Peroral inoculation of these cysts into fresh mice (10 cysts/mouse) produced acute lethal toxoplasmosis in one instance, and the recovered tachyzoites showed normal infectivity.
Conclusions: Early treatment of murine *T. gondii* RH strain infection with ATO and PDTC induces conversion of tachyzoites into tissue cysts, which in addition to showing therapeutic potential, provides a model for studying the mechanisms of stage conversion.

P1003 Investigation of congenital toxoplasmosis in *Rattus rattus*

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Background: *Toxoplasma gondii* is a coccidian parasite of felides and has, as intermediate hosts, many warm-blooded animals, including mammals and birds. Congenital toxoplasmosis is one of the most important infectious disease seen in fetuses and infants born from mothers infected with *T. gondii* during pregnancy. Congenital infections, which may occur of a mother is infected for the first time during pregnancy, is often serious, resulting in abortion or severe neurological and ophthalmological disorder. Information on human cases of neonatal toxoplasmosis makes it unquestionable that toxoplasma crosses the placenta and invades the fetus in utero.
Objective and methods: *T. gondii* infection in newborn rat litter was detected by a bioassay in mice. Rat's litter were killed and their tissues were separately hemogenized in normal saline or PBS and inoculated intraperitonealy in to 3 mice. The tissues used for bioassays were brain, hearts, lungs, livers, and spleen of pups, which were killed on the day of birth. In a study of congenital toxoplasmosis in rats during the acute stage of toxoplasma infection, three trails were performed, and the day of gestation at the time of *T. gondii* inoculation ranged from 6 to 18 (day 6, 12, and 18 of pregnancy).
Results: The results demonstrated that toxoplasma infection of rats on day 12 of pregnancy is the most effective time to cause congenital toxoplasmosis (with 62.5%), but the incidence of congenital toxoplasmosis in 18th day of pregnancy is 37.5%, and none of the 105 litters from 6th day of pregnancy indicate infections. Data on the occurrence of congenital transmission from chronically infected mother rats given similarly graded inoculums of the RH Strain ($1 \times 10^6 - 5 \times 10^6$) are presented that none of the 36 pups was infected with *T. gondii*. The occurrence of congenital transmission in rats which were reinfected with toxoplasma shows that none of the 14 pups was infected with *T. gondii*.
Conclusions: Thus, this study demonstrates that *Rattus* chronically infected with *T. gondii*, have immunity capable of protecting their embryos from congenital infection, even if they are reinfected during pregnancy.

Macrolides

P1004 Activity of telithromycin against macrolide-resistant *Streptococcus pyogenes* isolated from pediatrics: PROTEKT Year 1 vs. 2

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Background: Increasing erythromycin resistance (ERY-R; MIC $\geq$ 1 mg/L) among *Streptococcus pyogenes* is causing concern, particularly as macrolides are the main option for penicillin-allergic patients in the treatment of RTIs. Data from the PROTEKT study were analyzed to assess the prevalence of ERY-R among *S. pyogenes* isolated from pediatrics during the winters of 1999–2000 (Y1) and 2000–2001 (Y2) and to assess the activity of telithromycin (TEL) against such isolates.
Methods: *S. pyogenes* isolates were collected worldwide from children aged $\leq$14 years with community-acquired RTIs from 53 centers in Y1 and 58 centers in Y2. Antibacterial susceptibility of the isolates was assessed using NCCLS guidelines. Macrolide resistance mechanisms were determined by PCR in ERY-R isolates.
Results: A total of 676 (Y1) and 713 (Y2) isolates of *S. pyogenes* were collected from pediatric patients. The prevalence of ERY-R isolates was 10.2% ($n = 69$) in Y1 and 9.3% ($n = 66$) in Y2, with high cross-resistance to clarithromycin (66/69, 95.7% Y1; 63/66, 95.5% Y2) and azithromycin (69/69, 100% Y1; 66/66, 100% Y2). Mef(A) followed by erm(B) was the principal macrolide resistance mechanism in both years. TEL was highly active (MIC90 $\leq$ 0.03 mg/L) in both years. At $\leq$ 1 mg/L, TEL inhibited 96% (Y1) and 98% (Y2) of all isolates, including, for both years, all strains harboring mef(A) or erm(TR). Isolates harboring the *erm(B)* gene had a telithromycin MIC range of 0.015 to >32 mg/L (Y1) and 0.06–16 mg/L (Y2).

	No. of isolates											
	Y1						Y2					
Country	All	ERY-R	mef (A)	erm (B)	erm (TR)	Neg*	All	ERY-R	mef (A)	erm (B)	erm (TR)	Neg*
Italy	30	11	1	10	0	0	51	6	2	4	0	0
Japan	59	12	10	1	1	0	84	2	1	1	0	0
Portugal	34	10	3	7	0	0	2	1	0	1	0	0
Spain	0	–	–	–	–	–	80	22	18	2	1	1
Worldwide	676	69	37	28	4	0	713	66	38	18	8	2

*Countries with $\geq$10 ERY-R isolates in either year.
*Negative for resistance mechanisms tested.

Conclusions: mef(A)-mediated macrolide resistance among *S. pyogenes* from pediatrics is more prevalent worldwide than other macrolide resistance mechanisms. Telithromycin exhibits excellent activity against *S. pyogenes*, including most macrolide-resistant and all mef(A) isolates. Telithromycin activity is not reduced by mef(A) and hence it offers considerable potential for clinical utility in areas of macrolide resistance.

P1005 In vitro activity of telithromycin against *Streptococcus pneumoniae* isolated in Europe, Africa, Middle East and Asia during 2001

D. Felmingham, C. Janus on behalf of the E-BASKETT Study Group

Objective: Surveillance of antimicrobial susceptibility is fundamentally important as an aid for informed choice in the empirical therapy of infectious diseases. E-BASKETT, an international prospective study, determines the susceptibility of *Streptococcus pneumoniae* isolated from patients with community-acquired respiratory tract infections to established antimicrobial classes and to the new ketolide, telithromycin.
Methods: 1563 isolates of *S. pneumoniae* were collected in 23 countries from March 2001 and January 2002. The in vitro susceptibility of isolates to various antibacterials was determined by a disk diffusion method at the participating laboratories and by MIC determination following NCCLS guidelines at a central laboratory.
Results: The prevalence of penicillin G resistance (resistant [PEN G R] – MIC $\leq$ 2 mg/mL) and erythromycin A resistance (ERY A R – MIC $\leq$ 1 mg/mL) were as per table:

Country	*n*	PEN G-R (%)	ERY A-R (%)
Austria	187	3.2	9.6
Finland	90	4.4	14.4
Greece	72	41.7	48.6
Netherlands	63	0	3.2
Spain	108	28.7	37.0
Switzerland	99	2.0	7.1
Czech Republic	160	4.4	3.7
Hungary	46	21.7	39.1
Poland	108	9.3	15.7
Slovakia	49	28.6	34.7
Turkey	41	14.6	7.3
Algeria	39	25.6	28.2
Ivory Coast	12	0	8.3
Lebanon	47	19.1	21.3
Morocco	13	0	0
Senegal	3	0	0
South Africa	196	50.0	57.7
Tunisia	44	29.5	38.6
South Korea	28	57.1	89.3
Philippines	26	0	0
Taiwan	87	72.4	94.3
Thailand	24	50.0	66.7
India	21	0	0

Overall, 99.3% of the isolates of *S. pneumoniae* were inhibited by $\leq$0.5 mg/L and 99.9% by $\leq$1 mg/L telithromycin (MIC_{50}, MIC_{90}, MIC range; 0.008, 0.12, 0.004–4 mg/L, respectively).
Conclusions: *S. pneumoniae* isolates from different geographical regions all showed a high overall susceptibility to telithromycin. In penicillin and erythromycin resistant strains of *S. pneumoniae*, telithromycin showed significant activity.

P1006 Accuracy in determining telithromycin MIC values against *Streptococcus pneumoniae*: *E*-test vs. microbroth dilution

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Objectives: *Streptococcus pneumoniae* (SP) is a common cause of outpatient respiratory tract infections routinely treated with oral agents including macrolides and the new ketolide telithromycin. Macrolide-resistant SP are increasingly common while telithromycin resistance is exceptionally rare in SP. Accurate susceptibility testing is essential to document SP telithromycin activity. This study documents the effects of CO_2 on telithromycin MICs when determined by *E*-test methodology.
Methods: A total of 75 SP, 22 (29%) azithromycin susceptible and 53 (71%) azithromycin-resistant were selected from recent international surveillance trials. All *E*-test values were determined after incubation in 5% CO_2 while microbroth dilution panels were tested under ambient air conditions following NCCLS guidelines. Both microbroth dilution panels and *E*-test plates were inoculated using the same broth inoculum. Random colony counts confirmed inocula.
Results: *E*-test and broth dilution telithromycin geometric mean (GM) MICs and fold GM increases for ALL, macrolide sensitive and macrolide resistant SP were: All [0.108/0.037, 2.9], macrolide sensitive [0.022/0.006, 3.7] and macrolide resistant [0.228/0.079, 2.9]. Telithromycin *E*-test MIC90s for ALL isolates increases 4-fold compared with microbroth dilution SP MIC90s. However no susceptible to resistant categorical interpretative changes were documented.
Conclusions: *E*-test is a valuable diagnostic tool but when testing SP against telithromycin in a CO_2 environment, MICs increase. The data suggest caution when interpreting telithromycin MICs when using telithromycin *E*-test strips in a CO_2 environment.

P1007 Three-year surveillance of resistance to beta-lactam antibiotics and telithromycin among bacterial pathogens associated with community-acquired respiratory tract infections in Japan

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Objectives: Japan has a high usage of beta-lactam (BL) antibiotics, which has led to increasing BL resistance. This has been monitored for 3 years in community-acquired respiratory tract infections (CARTIs) in Japan, as part of the PROTEKT global surveillance study.
Methods: During 1999/2000, 2000/1 and 2001/2, isolates (*n*/year) of *Streptococcus pneumoniae* (308, 627, 816), *S. pyogenes* (120, 161, 182), *Haemophilus influenzae* (281, 442, 545) and *Moraxella catarrhalis* (122, 186, 248) were collected from patients in Japan with CARTIs and their susceptibilities determined using NCCLS breakpoints (bpt) to the standard PROTEKT panel of antibiotics plus cefditoren, cefdinir and cefcapene.
Results: Each year, susceptibility of *S. pneumoniae* to penicillin, cefuroxime, cefixime, cefaclor, cefpodoxime and cefdinir was <50%. Cefcapene (no bpt) exhibited similar low activity to cefpodoxime (MIC_{90} 4 mg/L). Cefditoren (MIC_{90} 1 mg/L, no bpt) and amoxicillin-clavulanate (AMC, MIC_{90} 2 mg/L, >96% S each year) were the most potent BLs, but both were >4-fold less active than telithromycin ($MIC_{90} \leq 0.25$ mg/L, $\geq$ 99.6% S). All *S. pyogenes* were susceptible to all BLs (all $MIC_{90} \leq 0.5$ mg/L). The prevalence of BL+ and BLNAR (BL–, ampicillin-resistant [MIC $\geq$ 2 mg/L]) *H. influenzae* was 9, 10 and 4, 7, 10%, respectively, over the 3 years. Cefditoren ($MIC_{90} \leq$ 0.12 mg/L, no bpt) was the most active BL. Each year, >82% of isolates were susceptible to cefdinir and ~99% isolates were susceptible to cefpodoxime and AMC. BL + *M. catarrhalis* exceeded 94% each year, decreasing susceptibility to cefuroxime (92, 87, 83%), cefprozil (75, 72, 71%) and cefaclor (89, 84, 74%). Cefditoren (MIC_{90} 0.03–0.06, no bpt) was the most active BL and all isolates were susceptible to AMC (MIC_{90} 0.25 mg/L) and cefdinir (MIC_{90} 0.5 mg/L). Each year, $\geq$99% of *S. pneumoniae* (MIC_{90} 0.25 mg/L) and *H. influenzae* (MIC_{90} 2 mg/L) were susceptible to telithromycin. All *M. catarrhalis* and >97% of *S. pyogenes* (all $MIC_{90} \leq 0.25$ mg/L) were inhibited by telithromycin at 1 mg/L.
Conclusions: Japan has high levels of BL resistance among *S. pneumoniae* and rising levels of resistance among *H. influenzae* and *M. catarrhalis*. Cefditoren exhibits low MIC90s against these pathogens. Cefdinir and cefcapene exhibit weaknesses in their spectrums of activity. Telithromycin, the first ketolide antibiotic, may offer an alternative to BLs for CARTI therapy in Japan because of its potent activity even against BL-resistant strains.

P1008 In vitro activity of the ketolides telithromycin and ABT 773 against *Staphylococcus aureus* and their potential to select for resistant mutants

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Macrolides are widely used in the treatment of respiratory tract and soft tissue infections where *Staphylococcus aureus* is considered to be one of the major pathogens. The rising incidence of infections caused by erythromycin-resistant *S. aureus* strains is a growing problem that is leading worldwide to an increasing need for new agents. The ketolides telithromycin and ABT 773 represent a new class of antimicrobial agents and could possibly be of great value in the treatment of infections caused by pathogens such as *S. aureus*.

Objectives: The aim of the present study was to determine the in vitro activities of telithromycin and ABT 773 against *S. aureus* and to examine the potential of these two ketolides to select for resistant mutants.
Methods: 100 clinical *S. aureus* isolates were analyzed including 20 erythromycin-susceptible, 40 erythromycin-inducible-resistant, and 40 erythromycin-constitutive-resistant strains. The MICs of telithromycin and ABT 773 were determined by the agar dilution method and compared with those of clindamycin, clarithromycin, azithromycin, and quinopristin/dalfopristin. Additionally, the ability of telithromycin and ABT 773 to select for resistant mutants was investigated by subculturing three erythromycin-susceptible and three erythromycin-inducible-resistant *S. aureus* isolates in the presence of subinhibitory concentrations of these two ketolides.
Results: In vitro, telithromycin and ABT 773 showed high activity against erythromycin-susceptible and erythromycin-inducible-resistant *S. aureus* strains, with MIC90 values ranging between 0.125 and 0.25 mg/L. The subculturing experiment, however, revealed that telithromycin and ABT 773 easily select constitutive-resistant mutants in macrolide-inducible-resistant isolates. The MICs increased to >128 mg/L already within 2 days of subculturing and were stable after 10 days of antibiotic-free passages.
Conclusions: The results of the present study show that a cautious and judicious use of telithromycin and ABT 773 is strongly recommended in order to maintain the valuable status of this class of antimicrobial. As ketolide-resistant mutants can be easily selected overnight by the exposure of inducible-resistant isolates to telithromycin and ABT 773, ketolides should not be deployed for the treatment of infections caused by staphylococci of this phenotype.

P1009 Comparative in vitro activity of Telithromycin (KETEK), macrolides, quinolones and beta-lactams against *S. aureus*, *S. pneumoniae* and *H. influenzae*

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Background: Telithromycin, a semisynthetic new ketolide, was compared with erythromycin A, azithromycin, clarithromycin, ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, moxifloxacin, cefaclor, cefuroxime, amoxicillin and amoxicillin/acid clavulanate against more than 1000 recent clinical isolates.
Methods: MIC's were determined by agar dilution (NCCLS).
Results: Against *S. aureus* methi.-resistant (MecA), telithro. (MIC_{90} 0.06 mg/L) was more active than macrolides tested (MIC_{90} 8 mg/L), oflox. and ciproflox. (MIC_{90} 2 mg/L). A decreased activity (MIC 4 mg/L) was observed with telithro. against erythro.-resistant or MLSb-resistant *S. aureus* strains. Against *S. aureus* ciproflox.-resistant pathogens, telithro. (MIC_{90} 0.006 mg/L) was the most active compounds tested, followed by gatiflox., moxiflox. (MIC_{90} 2 mg/L) and cefurox. (MIC_{90} 4 mg/L). Telithro. (MIC_{90} 0.5 mg/L) was superior to oflox. (MIC_{90} 2 mg/L), claritho. (MIC_{90} 256 mg/L) and cefurox. (MIC_{90} 0.5 mg/L) against *S. pneumoniae* peni.-resistant or peni.-intermediate strains. Against *S. pneumoniae* erythro.-resistant (ermB), telithro. (MIC_{90} 0.01 mg/L) was the most active agents tested. Against *S. pneumoniae* erythro.-resistant (mefE), telithro. moxiflox. and gatiflox. (MIC_{90} 0.5 mg/L) were the most active agents tested. Against *H. influenzae* Beta-lactamase- or erythro.-resistant strains, telithro. (MIC_{90} 4 mg/L) was more active than clarithro. and Beta-lactams tested.
Conclusion: Telithromycin (KETEK) could be a valuable compound for the treatment of respiratory pathogens, including those resistant to usual oral therapy.

P1010 In vitro activity of Telithromycin (KETEK) vs. macrolides, quinolones and beta-lactams against *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* isolated from adults and children specimens

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Background: Telithromycin, a new ketolide, was compared with clarithromycin, azithromycin, erythromycin A, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin, cefotaxime, cefixime, cefpodoxime, cefaclor, cefuroxime, amoxicillin and amoxicillin/acid clavulanate against recent *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* strains isolated from adults (18 years old) and three age groups of children (1–6, 7–12, 13–17 years old).
Methods: MIC's were determined by agar dilution (NCCLS).
Results: *H. influenzae* and *M. catarrhalis* strains isolated from adults or from children showed similar activity with all tested antibiotics. With *S. pneumoniae* strains, most macrolides and cephalosporins tested were more active against strains isolated from children than against strains isolated from adults. Telithro. (MIC_{90} 0.5 mg/L) was superior to macrolides tested (MIC_{90} 16 mg/L) against *S. pneumoniae* peni.-resistant strains isolated from adults. In children group, *S. pneumoniae* peni.-resistant strains were more susceptible to telithro. (MIC_{90} 0.06 mg/L). Against *S. pneumoniae* (ermB genotype) isolated from adults, telithro. (MIC_{90} 0.01 mg/L) was the most active agents followed by moxiflox. (MIC_{90} 0.25 mg/L) and gatiflox. (MIC_{90} 0.5 mg/L). Against *S. pneumoniae* (mefE genotype) isolated in children (1–6), telithro. (MIC_{90} 0.25 mg/L), moxiflox. (MIC_{90} 0.25 mg/L) and gatiflox. (MIC_{90} 0.5 mg/L) were the most active agents followed by levoflox. and cefotaxime (MIC90 1 mg/L). Against *H. influenzae* β-lactamase-positive or ery-resistant strains isolated in both group, telithro. (CMI_{90} 4 mg/L) was more active than clarithro. and more active than β-lactams tested. Against *M. catarrhalis* β-lactamase positive strains isolated in both group, telithro. (MIC_{90} 0.06 mg/L) was comparable to moxiflox., levoflox., oflox. and ciproflox. Against *M. catarrhalis* ery-resistant, telithro. and azithro. showed a similar activity (MIC_{90} 2 mg/L).
Conclusion: Combined with favorable pharmacokinetics in human, telithro. could be a valuable oral compound for the treatment of *S. pneumoniae* and *M. catarrhalis* isolated from adults or children, including resistant strains to usual oral therapy.

P1011 Comparative in vitro activity determination of ketolides, macrolides, and azalides against the spirochete *Borrelia burgdorferi*

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Objectives: So far, ketolides belonging to a new class of antimicrobial agents, have not been tested in vitro against larger numbers of the *B. burgdorferi* complex derived from different clinical sources and geographic origin. Here we analyzed the in vitro activity of new ketolides, ABT-773 and telithromycin, in comparison to that of four classical macrolides, one azalide derivative, apramycin, and ceftriaxone against borreliae.
Methods: Seventeen isolates of the *B. burgdorferi* sensu lato (s.l.) complex in addition to one *Borrelia valaisiana* and one *Borrelia bissettii* tick isolate were tested. Minimal inhibitory concentrations (MICs) and minimal borreliacidal concentrations (MBCs) providing 100% killing of the final inoculum were determined by a standardized colorimetric test methodology and conventional subculture. In addition, time-kill studies and electron microscope analysis were performed on borreliae exposed to increasing drug concentrations.
Results: The rank order of potency on a µg/mL basis for the substances tested against *B. burgdorferi* was ABT-773 (MIC_{90}: 0.002 µg/mL, MBC_{90}: 0.12 µg/mL) > telithromycin (MIC_{90}: 0.007 µg/mL, MBC_{90}: 0.25 µg/mL) > azithromycin (MIC_{90}: 0.01 µg/mL, MBC_{90}: 0.5 µg/mL) > clarithromycin (MIC_{90}: 0.03 µg/mL, MBC_{90}: > 0.5 µg/mL) > ceftriaxone (MIC_{90}: 0.03 µg/mL, MBC_{90}: 2 µg/mL) > roxythromycin (MIC_{90}: 0.06 µg/mL, MBC_{90}: > 0.5 µg/mL), erythromycin (MIC_{90}: 0.06 µg/mL, MBC_{90}: > 0.5 µg/mL) > apramycin (MIC_{90}: > 64 µg/mL, MBC_{90}: > 64 µg/mL). Results of electron microscope analysis and time-kill studies clearly support enhanced in vitro activity of the ketolides on borreliae.
Conclusions: Our findings emphasize superior in vitro effectiveness of the new ketolide antibiotics ABT-773 and telithromycin in comparison to classical macrolides against *B. burgdorferi* under strictly standardized test conditions and hence the possible suitability of these substances for clinical trials on their performance in the treatment of Lyme disease.

P1012 Agar dilution and *E*-test MIC values of azithromycin, chlarithromycin and erythromycin among *Haemophilus influenzae* isolates

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Objectives: To study the suitability of *E*-test for the quantitative susceptibility testing of *H. influenzae* for three antibiotics from the macrolide and azalide groups: azithromycin, chlarithromycin and erythromycin. As a reference method agar dilution was used instead of the broth microdilution method recommended by the NCCLS-standard (2000). Since quality control limits

have been defined by the NCCLS for only one of the macrolides; azithromycin, the novel ketolide, telithromycin was included. Telithromycin *E*-tests were not performed while the strips are not yet available.
Methods: 374 consecutive clinical *H. influenzae* isolates from seven clinical microbiology laboratories in Finland were tested according to the guidelines of the NCCLS-standard (agar dilution method for nonfastidious bacteria) and the manufacturer of the *E*-test method (Biodisk Ab). HTM-agar was used for both methods. *H. influenzae* ATCC 49247 was used as a quality control for both tests. The results were analyzed using the WHONET5.1-computer program.
Results and conclusions: Acceptable quality control limits for *H. influenzae* ATCC 49247 have been defined by NCCLS for azithromycin and telithromycin. The QC-results of these antibiotics using the agar dilution method indicate that it can be used as a reference method for the MIC of *H. influenzae* isolates instead of the broth dilution (NCCLS recommendation). The results fell, with one exception, within the acceptable QC-limits for the broth dilution method. Consequently, agar dilution was used as a comparative method for chlarithromycin and erythromycin, as well. The *E*-test method showed also QC-results for azithromycin within the accepted range given by the NCCLS. The MIC_{50}-values of the clinical isolates for erythromycin and chlarithromycin were lower with the *E*-test compared with agar dilution but the corresponding values for azithromycin were identical. The MIC_{90}-values and geometric means of the MIC-values were lower with the *E*-test method for all three antibiotics. Interpretive categorization errors (classified as very major, if a resistant strain was classified as susceptible) with the *E*-test were as follows: azithromycin very major errors 19/374 (5.1%), total errors 23/374 (6.2%); chlarithromycin very major errors 8/374 (2.1%), total errors 111/374 (30.2%); erythromycin very major errors 1/374 (0.3%), total errors 237/374 (63.4%). The possible causes for these observations will be discussed.

P1013 Time-kill kinetics of clarithromycin, roxithromycin, and azithromycin against selected *Streptococcus pneumoniae* isolates compared with those of penicillin and moxifloxacin

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Objectives: Macrolides are supposed to exert marginal concentration-dependent killing. Time-kill kinetics are observed for 6 h (h) and 24 h according to the German DIN and NCCLS criteria, respectively. This study aimed at investigating the kill kinetics of three macrolides, penicillin (PEN), and moxifloxacin (MOX) against *S. pneumoniae* (Sp) applying both the DIN and the NCCLS criteria.
Methods: Time-kill kinetics of clarithromycin (CLA), azithromycin (AZI), roxithromycin (ROX), PEN, and MOX were determined for 4 Sp strains (PEN- and CLA-susceptible (S) (Sp1), PEN-intermediate (Sp2), PEN-resistant (R) (Sp3), and CLA-R (SP4)). MICs (mg/L) were: CLA, 0.015–2; AZI, 0.06–4; ROX, 0.03–8; PEN, 0.004–2; MOX, 0.125. Antibiotics were tested at 1× MIC, 2× MIC and 4× MIC. Colony forming units were counted at 0, 2, 4, 6, 8, and 24 h. A bactericidal effect was defined as a decrease of the inoculum by 99.9% (3 log 10).
Results: Applying NCCLS criteria all antibiotics exhibited bactericidal activity at 4× MIC against all Sp strains. At 2× MIC only CLA and MOX showed a bactericidal effect against all Sp strains. Applying DIN criteria, bactericidal activity was achieved for all antibiotics against Sp1 but only for CLA and PEN against Sp2, Sp3, and Sp4. Among the macrolides the rate and/or extent of killing against Sp strains was highest for CLA.
Conclusions: All antibiotics exhibited bactericidal activity against *S. pneumoniae* according to NCCLS, but according to DIN criteria only CLA and PEN were bactericidal. Bactericidal effect of CLA against Sp strains was stronger than that of AZI and ROX and comparable to that of PEN and MOX.

P1014 Different susceptibilities of implant-associated bacteria against azithromycin and β-lactam antibiotics in mixed and pure culture

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Objectives: Reduced susceptibility to β-lactam antibiotics in mixed culture has been described for abdominal pathogens. For the oral situation a symbiosis between Veillionellae and Lactobacilli is recognized. However no data is available regarding antimicrobial resistance.
Methods: During routine follow up microbial specimens were drawn from the periimplant pocket of 19 patients using sterile paper tips. The samples were diluted in sterile saline and cultivated under anaerobic conditions on BHI and Schaedler agar for 7 days. After cultivation, the mixed cultures were resuspended again in saline and mixed with soft agar of the respective medium as top agars. *E*-test strips containing azithromycin (AZI), ampicillin (AMP), ampicillin + sulbactam (AMP-SUL) and penicillin G (PEN) were placed on the top agar and the plates were incubated for >24 h under anaerobic conditions. After reading the *E*-test, resistant strains were picked and purified and the *E*-test was repeated with the pure isolate.
Results: Mean values of the MIC (μg/mL) of the mixed cultures (BHI and Schaedler) and of the respective isolates are given:

	AZI	AMP	AMP-SUL	PEN
BHI mixed ($n = 19$)	230	167	174	24
Schaelder mixed ($n = 19$)	206	249	212	30
Isolates ($n = 103$)	68	13	76	12

Conclusion: No relevant difference of the MIC values of periimplant pathogens in mixed culture was found using different culture media. However, after isolation lower MIC values were found for all antibiotics. Regarding the clinical situation of perimplantitis, he polymicrobial infection with its possible implications for antimicrobial therapy should be further elucidated.

Alternative agents

P1015 Activities of crude extracts of Thai medicinal plants on methicillin-resistant *Staphylococcus aureus*

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Objectives: Over recent years, there has been a global increase in the prevalence of antibiotic-resistant *Staphylococcus aureus*. The aim of our study was to screen for effective medicinal plants, widely used in Thai traditional medicine for the treatment of this organism.
Methods: Sixteen preparations of aqueous and ethanolic extracts of 10 kinds of Thai herbs including *Acacia catechu*, *Garcinia mangostana*, *Impatiens balsamina*, *Peltophorum pterocarpum*, *Psidium guajava*, *Punica granatum*, *Quercus infectoria*, *Tamarindus indica*, *Uncaria gambir*, and *Walsura robusta* were tested for their antibacterial activity against 35 hospital strains of methicillin-resistant *S. aureus* (MRSA) and *S. aureus* ATCC 25922. Inhibition of growth was preliminarily tested by the paper disc agar diffusion method. Antibiotic susceptibility discs were used as control. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the agar dilution method in Petri dishes with millipore filter.
Results: Nearly all extracts, except of *T. indica*, showed some antibacterial activity against all strains of MRSA and *S. aureus*. *P. granatum* and *Q. infectoria* showed the greatest inhibition zones, ranging from 13 to 23 mm. Among the plants tested, aqueous and ethanolic extracts of *Q. infectoria* and ethanolic extract of *P. granatum* and *G. mangostana* were demonstrated to be significantly effective with the MIC values of 0.05–0.4, 0.05–0.1, 0.2–0.4 mg/mL and the MBC values of 0.1–0.4, 0.1–0.4, 0.4–3.2 mg/mL, respectively.
Conclusions: As both aqueous and ethanolic extracts of *Q. infectoria* were very effective against all strains of MRSA, this plant should be further investigated for alternative treatment of this antibiotic-resistant organism.

P1016 Investigation into the antibacterial and immunomodulating effects of Lingzhi (*Ganoderma lucidum*)

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Introduction: Lingzhi (*Ganoderma lucidum*) is widely used in traditional Chinese medicine in the treatment of infections. There have been limited reports of its antimicrobial properties, though several authors have shown it to have modulating effects on the immune system.
Objectives: To investigate the antibacterial properties and effects on lymphocyte levels of Lingzhi extract.
Methods: A hot water extract from certified Lingzhi and a solution of a commercial source of Lingzhi were tested for their antimicrobial properties against a range of Gram-positive and Gram-negative organisms by means of broth dilution. Several strains of each organism including antibiotic sensitive and resistant strains were tested. Ten subjects were given three capsules of a commercial extract of Lingzhi or a placebo for 28 days. The treatment was followed by a 21-day wash out period and then subjects were crossed over to receive the alternative treatment. Fasting blood was collected on days 1 and 29 of each treatment period. Levels of CD3+, CD4+ and CD8+ cells were determined by flow cytometry.
Results: Lingzhi was found to have no antibacterial effect on *Staphylococcus aureus*, *Enterococcus*, *E. coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Salmonella typhi*, *Shigella*, *Serratia marcescens*, *Pseudomonas aeruginosa* or *Proteus mirabilis* even at a concentration of 16 mg/mL. Both penicillin sensitive and penicillin resistant *Streptococcus pneumoniae* were inhibited at 16 mg/mL. CD4+ and absolute CD4+ cells were significantly increased, 12% ($P < 0.05$) and 37% ($P < 0.01$), respectively. The increase in CD4+ cells led to a 25% increase in the CD4/CD8 ratio ($P < 0.05$).
Conclusions: Lingzhi appears to exert in vivo immunomodulatory effects resulting in an increase in CD4+ lymphocytes. This increase in CD4+ cells may indicate enhancement of CD4 receptors on T cells by MHC class II molecules on antigen presenting cells, which may help to further amplify cell-mediated response to exogenous pathogens. In contrast to a previous report, Lingzhi did not appear to demonstrate antibacterial activity in vitro, except at high concentration for *S. pneumoniae*. The success of Lingzhi in the treatment of infection is likely to be due to its immune regulating function rather than its specific antibacterial effects.

P1017 The antimicrobial properties of the essential oil of *Melaleuca alternifolia* (tea tree oil) and its individual components

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Objectives: To examine the antimicrobial properties of tea tree oil and identify those components that possess antimicrobial activity by testing against a range of organisms.
Methods: Clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), *Acinetobacter junii* and *Candida albicans* were tested against tea tree oil and eight of its components using time kill curves. The components tested were alpha-terpinene, gamma-terpinene, terpinolene, cymene, linalool, alpha-terpineol, terpinen-4-ol and 1,8-cineole at various concentrations.
Results: All the microorganisms tested proved susceptible to tea tree oil. *C. albicans* was the most susceptible, and VRE was the least susceptible. Of the tea tree oil components tested, alpha-terpinene, gamma-terpinene, terpinolene and cymene showed no or negligible antimicrobial activity. 1,8-cineole showed antifungal activity against *C. albicans*, and antibacterial activity against *A. junii*, but no antibacterial activity against MRSA or VRE. Linalool, alpha-terpineol and terpinen-4-ol showed antimicrobial activity against all the organisms tested, with alpha-terpineol shown to possess slightly greater antimicrobial activity against VRE and MRSA than terpinen-4-ol (the main antimicrobial component in tea tree oil).
Conclusion: Alpha-terpineol could be considered as an alternative to tea tree oil and conventional antimicrobials in the topical treatment of infection. The use of individual components of tea tree oil may eliminate the potential for allergic reactions often associated with tea tree oil.

P1018 Time kill curves of tea tree oil and sweet marjoram against MRSA and *E. coli*

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Objective: There have been minimal studies carried out on the rate of bacterial cell growth or death in the presence of essential oils (EOs). It was, therefore, decided to carry out time kill curves on tea tree oil and sweet marjoram which have both shown antimicrobial activity
Methods: The bacteria, *Escherichia coli* (*E. coli*) and methicillin-resistant *S. aureus* (MRSA) used in the following experiments were obtained from clinical isolates. The EOs studied included *Melaleuca alternifolia* (tea tree) and *Origanum majorana* (sweet marjoram). Tea tree oil (TTO) and sweet marjoram were doubly diluted in nutrient broth to a final concentration ranging from 10 to 0.08% (v/v). Time kill curves were then carried out on 0.30 and 0.08% TTO and sweet marjoram against MRSA and *E. coli*.
Results: At 0.30% EO sweet marjoram reduced bacterial numbers to zero within 30 min whilst a similar reduction in bacterial numbers required 8 h with TTO. At the lower concentration of 0.08% both EOs were not bactericidal to either MRSA or *E. coli* after 8 h incubation
Conclusions: Although the majority of research has centered on the antibacterial properties of TTO and its uses against MRSA, the present experiments have shown that Sweet marjoram has greater antibacterial properties than TTO against MRSA and *E. coli*. Sweet marjoram is less irritant to skin when applied topically at concentrations of 1–30% and has lower oral toxicity when compared with TTO. This could be used as a viable alternative to TTO in the eradication of MRSA.

P1019 Antibacterial efficacy of commercially manufactured disinfectant substances against *Salmonella enterica* serovar Enteritidis

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Objectives: To evaluate the antimicrobial efficacy and effect on the biosynthetic processes as well as on the endogenous respiration of 19 commercially manufactured disinfectants on *Salmonella enterica* serovar Enteritidis.
Methods: The antimicrobial efficacy was characterized by influencing the growth of bacterial cells expressed by MIC and ED_{50} values as well as by the inhibition of the incorporation rate of [^{14}C]adenine and [^{14}C]leucine into TCA-insoluble fractions of cells. The endogenous respiration was determined polarographically with a Clark type oxygen electrode.
Results: The disinfectant substances represented eight quaternary ammonium salts (QAS) and 11 combinated QAS with other ingredients. These substances were divided into three groups according to their efficacy. The first group comprised the substances with a strong inhibitory effect (MIC 0.006–0.045 μg/mL) such as Diesin forte, Hexaquart plus, Neoquat S, Triquart, Almyrol, Hexaquart S, ID212, ID213 and Microbac forte. The second group represented substances with a good antibacterial efficacy (MIC 0.09–0.78 μg/mL) and in the third group were substances with MIC values up to 0.78–3.12 μg/mL. Cetrimide had a very low activity (MIC 3.12–6.25 μg/mL). The effect of disinfectants on the biosynthetic processes expressed by *R*-values (IC_{50}Ade:IC_{50}Leu) showed that these values were ~1 (except for six substances), which suggested interference of these substances with cellular energy metabolism. All substances except 5Pplus caused an inhibition of the endogenous respiration of *Salmonella* cells.
Conclusions: The results suggest that these techniques can be used for evaluation of antibacterial efficacy and demonstrate the mechanism of action of disinfectant substances.

P1020 The effect of human lactoferrin on the antibiotic susceptibilities of *Stenotrophomonas maltophilia* isolated from cystic fibrosis patients

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Objectives: The presence of lactoferrin at the high concentration (0.9 mg/mL) found in the respiratory secretions of cystic fibrosis (CF) patients has

effects on bacterial growth and may affect the susceptibility of CF pathogens. The effect of lactoferrin at this high concentration on the susceptibility of Rifampicin, Gentamicin, chloramphenicol, erythromycin, Ceftazidime and Trimethoprim against *Stenotrophomonas maltophilia* obtained from CF patients were investigated.
Methods: MICs for the clinical isolates of *S. maltophilia* in the presence and absence of lactoferrin (0.9 mg/mL), were tested by a microtiter broth dilution method. MBCs were also determined. For each clinical isolate, eight replicate MIC and MBC determinations were performed per antibiotic. MICs and MBCs quoted are median values.
Results: The presence of lactoferrin lowered the MICs and MBCs of rifampicin by 2–16-fold for clinical isolates of *S. maltophilia.* There were no changes in the susceptibility of the other antibiotics when lactoferrin was added.
Conclusions: These findings may be of relevance to the treatment respiratory tract infections in CF patients, in whom the presence of lactoferrin in vivo may enhance the activity of antibiotics, an effect that would not be predicted by standard antibiotic testing methods. In this setting it may be useful to incorporate lactoferrin in antibiotic sensitivity testing methodology.

P1021 In vitro activity evaluation of some antibiotics included in a hydrogel

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Background: Latest years revealed the benefits of local therapy in dermatological infectious affections. Good local tolerance of tested hydrogel encouraged us to elaborate four formulations containing well known antibiotics, used in dermatology.
Objectives: We intended to evaluate in vitro activity and stability of antibiotics included in our hydrogel.
Material and methods: We used a bentonite hydrogel prepared after an original method which met the European Pharmacopoeia's quality conditions. We included in hydrogel sodium benzylpenicillin, chloramphenicol, tetracycline hydrochloride and neomicine sulfate. The products were characterized physic-chemically. The antibiotic activity was evaluated using difusimetric methods. As test microorganisms we used *Staphylococcus aureus* 6538 P and *Escherichia coli* ATCC 10536.
Results: The hydrogel pH is 7.0, and the pH of antibiotic containing hydrogel varies with each antibiotic. Benzylpenicillin hydrogel had the lowest stability, but the chloramphenicol hydrogel and neomicine sulfate hydrogel maintained the activity throughout the study time (98 days). Benzylpenicillin hydrogel had a mild activity decrease after 19 days (82%) and only 48% of initial activity after 30 days. Tetracycline hydrochloride hydrogel maintained the initial activity 30 days, but only after 48 days the activity become 95% of initial one, and remained so until the end of study. Moreover we studied the influence of surfactants on antibiotic release from hydrogel.
Conclusions: *E. coli* was more sensitive to the tested antibiotics than *S. aureus*. Benzylpenicillin hydrogel had the lowest stability. Surfactants use increased the antibiotic stability included in hydrogel.

P1022 'Ex vivo' synergism: a method to optimize antimicrobial combinations for difficult-to-treat endocarditis

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Pisa, I

We describe a method to evaluate synergism between the antibacterial activity of patient serum taken during a given antibiotic combination added in vitro with further antibiotic. A 63-year-old male patient was admitted to cardiac unit with a mitral valve endocarditis due to *E. faecalis*. The blood isolate resulted susceptible but tolerant to penicillin, ampicillin, teicoplanin and vancomycin; highly resistant to gentamycin, tobramycin, and streptomycin and susceptible to netilmicin. No beta-lactamases production was found. The patient was treated with penicillin (24 MU/day) and netilmicin (150 mg t.i.d.), a combination resulted fully synergistic with the checkerboard method (SFICI: 0.2). After 4 days of therapy, peak and through serum bactericidal activity (SBA) were both 1:16 and blood cultures were still positive for *E. faecalis*. Therefore, we tested synergism between patient serum and teicoplanin with the checkerboard method. The combination of the peak serum with teicoplanin gave a SFICI of 0.02 indicating fully synergism. Teicoplanin was therefore added to the current patient treatment. After 4 days peak and through SBA resulted 1:128 and 1:32, respectively. Blood cultures became negative on day 8 and endocarditis was cured after 4 weeks of the three drugs combination therapy. Ex vivo synergism between the patient serum during antibiotic therapy and further antibiotics might be an useful method to optimize the antibiotic therapy in difficult-to-treat infections.

Does antibiotic policy affect usage?

P1023 Is community antibiotic prescribing consequence-free?

J. T. Magee, J. L. Bell, M. L. Heginbothom, A. J. Howard – Welsh Antibiotic Study Group

Objectives: To elucidate factors associated with antibiotic resistance in community infection as a guide to rational intervention.
Methods: Retrospective resistance data on community isolates and antibiotic prescribing data for 1996–2001 were collected for Wales. Analyzes were performed in Excel and SPSS.
Results: During the study, overall prescribing decreased by *c.* 40% in Wales. Antibiotic prescribing varied markedly between practices, with a minority prescribing up to four to five times the mean. Similarly, resistance levels varied, sometimes markedly between practices. For *Staphylococcus aureus* and urinary coliforms there was a clear association between high practice prescribing of an antibiotic and high levels of resistance to that antibiotic. There was also an association between resistance and social deprivation exceeding that expected from high prescribing in deprived areas. The proportion of resistant infections appeared to be highest in the young (<10 years) and the aged (>70 years). This was a generalized rule across a wide variety of infections (urinary, respiratory and wound) and pathogens (urinary coliforms, *H. influenzae*, MSSA, MRSA, pneumococci and group A streptococci). Community methicillin resistance in *S. aureus* rose linearly from age 25, and showed a more rapid increase beyond age 70. The age-resistance distributions for pooled data from practices in the highest quartile of prescribing generally showed curves parallel to those for pooled data from the lowest quartile, with significantly higher resistance throughout the age range. For some infections and some antibiotics, resistance was significantly greater in males.
Conclusions: Antibiotic usage appears to have consequences on resistance levels in community infection at practice level. The statistical link between practice usage and practice resistance documented previously for urinary coliforms has been confirmed and extended to infections for another pathogen with distinct pathology and epidemiology – *S. aureus*. This association also appears to operate throughout the age range of underlying pathologies involved in coliform urinary tract infections. Other factors associated with increased resistance were patient age and gender. Possible mutual associations between age, prescribing and resistance deserve further investigation.

P1024 Statistics and resistance: is guessing really easier than this?

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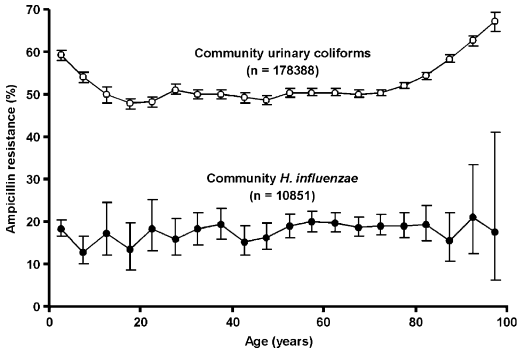
Objectives: To develop methods for preliminary analysis of an all-Wales survey of community antibiotic resistance (*c.* 350 000 isolates). Automated methods were needed to show the level of uncertainty for individual resistance estimates (REs) and in trend and scatter graphs, and to compare large numbers of REs objectively.
Methods: The confidence interval is regarded as essential in presentation of numerical data by many medical journals. The CI indicates the plausible range

of values for the true level consistent with the data, taking into account variation from random sampling effects (e.g. a RE of 0.1% based upon 1000 isolates has an uncertainty from *c.* 0.02 to 0.56%; the single resistant isolate might represent chance inclusion of rare strain, or chance exclusion of more common strains in the sample). For REs, approximation CI formula found in books for nonstatisticians are not generally valid as they assume resistance not close to 0 or 100% and large samples. Formula allowing rigorous calculation of CIs for REs, and for comparisons between REs were written in Excel spreadsheets and utilized in preliminary analysis of data from the All-Wales survey. Detailed methods for incorporation of these formula into Excel spreadsheets and production of graphical representations that include error bars will be available.

Results: Microbiologists' intuitive estimates of uncertainty were often grossly optimistic. The small effort of incorporating the formule into a general-purpose pro-forma analysis spreadsheet aided interpretation. Ready visualization of the range for estimates facilitated interpretation of trend and scatter plots, and where sample sizes were widely variant, e.g. for interpractice and interlaboratory comparisons. In one analysis, CIs of REs for 13 antibiotics against six species at 14 laboratories were calculated and compared with all-Wales REs. Excel sorting of the comparison CIs listed laboratories in order of statistical deviation from the all-Wales estimate. This ideal presentation of data in analysis priority order took less than 10 min to achieve.



Conclusions: The Excel formule are convenient and allow much more rigorous interpretation of resistance data than normally occurs. They are not a panacea for all statistical problems – advice from an experienced statistician should always be sought. CIs are a vital adjunct to interpretation of susceptibility data, and essential in published papers.

P1025 Audit of linezolid use within a large teaching NHS trust

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Objectives: We wished to audit the frequency, distribution, and indications for linezolid use within Lothian University Hospitals, NHS Trust for the 12 months from the 1st November 2001 to the 31st October 2002.

Methods: The Trust covers three major hospital sites and includes tertiary referral centers. A protocol for use of Linezolid was agreed and put into place in November 2001. The protocol stated that linezolid was only prescribed with the approval of a consultant microbiologist or infectious disease physician for one of four indications: (1) organisms resistant to conventional antibiotics, (2) patient intolerant to vancomycin, (3) intolerance of other oral therapy for MRSA, and (4) failed glycopeptide therapy. The pharmacist for each clinical area was asked to complete an audit form for each course of therapy. Pharmacia kindly provide monthly data on the amount of linezolid purchased by the Trust.

Results: Audit forms were completed for a total of 48 patients. The forms stated that 26 patients (54%) were prescribed linezolid for failed glycopeptide treatment, 8 because of intolerance of other oral therapy, and 16 because of infections resistant to conventional therapy. 23 had infection with MRSA, and 12 with VRE. The length of treatment was recorded for 29 patients, and ranged from 2 to 42 days with an average of 10 days. The cost of linezolid accounted for by the audit was £37 706, assuming and average course of 10 days for all patients. The total expenditure on linezolid over the same period was £74 369.

Conclusions: Large amounts of money are being expended on linezolid. Our audit data accounts for only about half of all the linezolid purchased. The use on 'hard' indications (VRE, glycopeptide intolerance, and intolerance of oral MRSA regimens) has been roughly as we had anticipated, but the expenditure on the 'soft' indication of glycopeptide failure has been greater than expected and seems to be increasing. There is a need to develop better audit processes if we are to understand and manage the use of this valuable drug.

P1026 Audit of usage of i.v. antibiotics in an Irish hospital, Dublin for a 4-week period

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Introduction: In keeping with the Strategy to Control Antimicrobial Resistance in Ireland, empiric antibiotic guidelines were developed and launched in St. James's Hospital (SJH) in November 2001. 9 months post introduction of the guidelines an audit of acceptance of these guidelines was undertaken.

Methods: Data was collected within 24 h of i.v. antibiotic commencement for a 4-week period. Parameters recorded included patient specific data, indication for antibiotic, antibiotic-specific data, microbiology data and intervention data.

Results: The patient cohort consisted of 200 patients (45% females, 55% males; 58% medical and 42% surgical patients). Empiric antibiotic therapy was prescribed for 91% of patients. For some conditions such as febrile neutropenia ($n=7$), bacterial meningitis ($n=2$) and skin and soft tissue infection ($n=41$), acceptance of the SJH empiric antibiotic guidelines was excellent (90–100%). For other clinical conditions such as community acquired pneumonia ($n=52$) and abdominal infection ($n=23$), acceptance of institutional guidelines was considered good (60–90%). However, poor guideline acceptance (<60%) was seen in conditions such as hospital acquired pneumonia ($n=25$) and bacteremias ($n=2$). Microbiological data indicated that 27.5% of all patients prescribed i.v. antibiotics had no specimens taken. Of the specimens taken, 70% of patients yielded positive microbiological data and these patients received appropriate tailored antibiotic therapy. Despite the suggestion of interventions by combined Infectious Diseases, Clinical Microbiology and Clinical Pharmacy services for 48% of patients, 31% of interventions were rejected. Rejections consisted of using antibiotics with over-lapping spectra in the absence of microbiological data and poor understanding of the switch from intravenous to oral administration.

Conclusion: The audit has highlighted areas (disease and specialty-specific) where concordance of prescribing with the EAG has been almost universal. However, some specific areas were identified where antibiotic prescribing was significantly at odds to the EAG, usually in the setting where minimal microbiological work-up was undertaken. The primary reasons for rejection of multidisciplinary suggested interventions related more to educational aspects of anti-infectives rather than to the EAG themselves. This further highlights the need for the establishment of continued educational programs focusing on appropriate antimicrobial prescribing.

P1027 Trends and errors of antimicrobial usage in a tertiary hospital

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Objective: Aiming intervention for proper antimicrobial (A) usage in our hospital, during a nationwide nosocomial prevalence study (04/06/2002) we also recorded antimicrobial usage to database

Methods: Prevalence recording study. As restricted A we define the ones that require a predefined application sheet to be filled. The A administration was considered justified if the recording physician was convinced by patient records or discussion with the attending physician/surgeon. Statistical analysis by nonparametric methods.

Results: On the day of recording, in our hospital departments of adults, excluding ICUs, a total of 357 patients, 145 (40.6%) medical and 212 (59,4%) surgical, were present. On A regimen were 177/357 (49.6%) patients, namely 66/145 medical (45.5%) and 111/212 surgical patients (52.4%) Restricted A had been prescribed to 58 (32.8%), 26 (39.4%) medical and 32 (28.8%) surgical ($P=$ NS). On more than one A were 66 (37.3%) patients, 31.8% of medical and 40.5% of surgical ones ($P=$ NS). Finally, the A administration was considered justified in 85 (48%) patients, 44 (66.7%) medical and 41 (36.9%) surgical (chi-square = 13.93567, $P<0.01$) ones. The main reason of

improper usage in surgical patients was a loosely termed 'chemoprophylaxis', not deemed by choice or duration, while the medical increased percentage was attributed to a sole department administering A, as a 'diagnostic criterion.' There were considerable differences among departments regarding the studied parameters.
Conclusions: This recording pinpoints the problem and allowed us to understand the points that must be targeted to achieve a more rational usage of antimicrobials in our hospital.

P1028 Bugs in the data 1: effects of testing policies and sampling on antibiotic resistance estimates

M. L. Heginbothom, J. T. Magee, J. L. Bell, A. J. Howard – Welsh Antibiotic Study Group

Objectives: To elucidate the effects of laboratory antimicrobial susceptibility testing (AST) policies and sample submission behavior on estimates of community antibiotic resistance.
Methods: Retrospective resistance data on routine community isolates for 1996–2001, specimen submission rates and practice list sizes were collected for Wales. Analyzes were performed in Excel and SPSS.
Results: Selective testing policies were often associated with resistance estimates markedly higher than those from laboratories that tested all isolates. These differences were often larger (in one case, 62% resistance compared with 2% resistance in nonselective testing) than might reasonably be explained by geographical variation. The effects were particularly marked where antibiotics were selectively tested as second-line agents on resistant isolates, or only when noted on the request form as being current therapy. Smaller (but often significant) effects were seen for site-specific testing. The majority of marked effects occurred where the laboratory performed AST on less than 20% of isolates of a species for an antibiotic. Comparison of practice resistance estimates with urine specimen submission rates per 1000 registered patients showed little correlation, despite wide variation in submission rates.
Conclusions: Selective laboratory AST policies can produce heavily biased resistance estimates. Most of this bias can be eliminated by removal of data where less than 20% of isolates for a specific antibiotic/species combination are tested by a laboratory. Bias due to variation in specimen submission policies seems to be consistent between practices, with no trend to increased resistance for practices with low specimen submission rates that might indicate selective sampling in, e.g. treatment failure. This consistency suggests that resistance prevalence estimates might be derived from routine data by application of correction factors deduced from regular sentinel studies. Our investigations of putative biasing effects suggest that, where bias can be detected, it is generally towards over-estimation of resistance. It may be possible to produce sets of rules and correction factors to remove these biases. In the interim, we regard resistance estimates from routine data as useful in comparative analyses of, e.g. variation in time or between practices, rather than prevalence figures. Nonetheless, these comparative analyses can be powerful tools in studies of resistance epidemiology.

P1029 Bugs in the data 2: effects of identification, repeat and screening isolates on resistance estimates

M. L. Heginbothom, J. T. Magee, J. L. Bell, A. J. Howard – Welsh Antibiotic Study Group

Objectives: To elucidate the effects of species identification policies for urinary coliforms, repeat isolates, and screening isolates on estimates of community antibiotic resistance from routine data.
Methods: Retrospective resistance data on routine diagnostic community isolates for 1996–2001 were collected for Wales and analyzed in Excel and SPSS.
Results: Laboratory policy on identification of urinary coliform isolates varied between the 14 participating laboratories. Isolates identified biochemically (minimum of indole test and lactose fermentation) as *Escherichia coli* were significantly less resistant than those identified as *E. coli* by colony morphology, and both were significantly less resistant than urinary coliforms with no species identification. For all species investigated, exclusion of repeat isolates (same patient, same susceptibility pattern ignoring mismatches for intermediate or not-tested results) produced negligible differences in resistance estimates. Exclusion of MRSA isolates from specimens overtly submitted as carriage screens, and from sites normally associated with carriage rather than infection, produced negligible differences in susceptibility estimates for MRSA. However, exclusion of repeat and screening isolates affected estimates of incidence, and MRSA screening isolates affected estimates of methicillin resistance in *Staphylococcus aureus*.
Conclusions: Species identification policies for urinary coliforms affect resistance surveillance. The identification requirements of the BSAC methodology; the implications of non-*E. coli* urinary infection on the underlying pathology for individual patients, and these surveillance effects may justify routine discrimination of *E. coli* from other urinary coliforms. Repeat and screening isolates have little effect on community resistance estimates, but inclusion of screening isolates of MRSA affects both methicillin-resistance estimates for *S. aureus*, and community MRSA prevalence estimates. It appears unnecessary to remove duplicate isolates for community resistance surveillance, and simple rules based upon specimen site can be used to eliminate screening isolates, removing this source of bias.

P1030 Microbiological consequences of a community-acquired pneumonia prophylaxis with azithromycin in military conscripts

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Background: Community-acquired pneumonia is a common cause of morbidity (230‰) in Russian military training camps. Prophylaxis with azithromycin is an effective method of outbreak control. But it's commonly accepted that high level of macrolide consumption correlates with macrolide and, sometimes, clindamycin and streptogramin B resistance of *Streptococcus pneumoniae*
Aims: To assess the carriage of macrolide-resistant pneumococci after azithromycin prophylaxis of CAP outbreak in military conscripts we conducted this prospective study.
Materials and methods: Two prophylaxis schemes with azithromycin vs. control (group 3) were evaluated: 8-week consumption of 500 mg/w (group 1), and once-though sanation with 1500 mg (group 2). Nasopharyngeal carriage of *S. pneumoniae* and its susceptibility to erythromycin, azithromycin, miocamycin, clin-damycin, penicillin, amoxicillin, cefotaxime, tetracycline and cotrimoxazol evaluated trice by broth microdilution: before treatment and after treatment on weeks 9 and 20.
Results: *S. pneumoniae* carriage rate at visit 0 was 34–43%; on week 9 in groups 1, 2, and 3 – 75, 66 and 50% ($P < 0.05$); on week 20 – 69, 57, and 36% in the same groups ($P < 0.05$). At visit 0 no macrolide resistance were detected in all 40 strains tested. But background level of intermediate penicillin resistance estimated in 0–14% of strains. Dramatic growth of macrolide resistance had been detected on 9th week. In the 1st group – 95.7% (44 resistant strains, 37% of them were moderately resistant to clindamycin and azithromycin) and in the 2nd group – 89.5% ($n = 34$, 11.9% moderately resistant to clindamycin and azithromycin). As for penicillin, we didn't find unfavorable resistance shift. By week 20th resistance rate in group 1 decreased up to 40% ($n = 16$, 10%) and up to 22.6% ($n = 7$, 5.4%) in group 2.
Conclusion: Prophylactic courses of azithromycin correlates with carriage of pneumococci and macrolide-resistant pneumococci. It is not revealed influences of both regimens on penicillin resistance. It is, therefore, important to continue to monitor macrolide resistance rates on a national level so that if prevalence rates begin to increase significantly, attempts to control their use should be made.

P1031 Macrolide-resistant *Streptococcus pneumoniae* in Canada: correlation with azithromycin use

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Objectives: Several investigators have suggested that the pharmacokinetic (long half-life, low and prolonged concentrations in serum, epithelial lining fluid and middle ear fluid), and pharmacodynamic (poor killing of macrolide-resistant *Streptococcus pneumoniae* [Sp] possessing the efflux phenotype), properties of azithromycin result in the preferential selection of macrolide-resistant Sp compared with macrolides such as clarithromycin and erythromycin. Here, we correlate regional differences in macrolide resistance in Sp from Canada with the use of azithromycin, clarithromycin and erythromycin.
Methods: Sp were collected from all provinces across Canada through national surveillance programs from both respiratory and sterile sites. Susceptibility testing was performed using NCCLS approved microbroth dilution techniques. Macrolide-resistant Sp were further characterized phenotypically and genotypically using a double disk diffusion technique and PCR for the

mef and erm genes. Provincial macrolide usage data was collected through IMS Canada and normalized for population.
Results: Macrolide resistance in Sp varied considerably in Canada in 2002, although three distinct trends were found. The coastal provinces demonstrated low rates of resistance (~5%), similar to rates previously described in 1995. The prairie provinces and Ontario demonstrate rates varying between 9 and 14%, while Quebec and the maritime provinces have rates exceeding 20%. The consumption of azithromycin in the coastal provinces remains the lowest in Canada accounting for <20% of prescribed macrolides. In contrast, azithromycin accounts for >44% of all macrolides in the three provinces with the highest rates of resistance. Use of azithromycin accounts for between 25 and 32% of total macrolide use in the prairie provinces. No correlation was found between total consumption of macrolides and the regional differences in resistance.
Conclusions: The prevalence of macrolide-resistant Sp varies considerably in Canada from, ~5 to >20%. Regions with the lowest rates of resistance use significantly less azithromycin vs. other macrolides while regions with the highest rates of resistance use more azithromycin vs. other macrolides. Our data suggest that azithromycin may have a greater propensity to select for macrolide-resistant Sp, compared with either clarithromycin or erythromycin.

P1032 Clinical audit in surgical units of a tertiary care hospital: antibiotic resistance profiles and commonly prescribed antibiotics. It's time for routine ESBL testing

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Introduction: Nosocomial infections with multiply antibiotic resistant organisms cause significant morbidity and mortality. Extended spectrum beta lactamase (ESBL) producing aerobic Gram-negative rods (AGNR) are resistant to second and third generation cephalosporins and monobactams, and may show simultaneous resistance to quinolones and aminoglycosides. They are known to have caused hospital outbreaks and are an important nosocomial infection issue. A clinical audit was carried out in the surgical units of Western Infirmary and Gartnavel General Hospital, Glasgow.
Objective: The aim was to establish the degree of correlation between commonly prescribed antibiotics in these surgical units and the antibiotic resistance profile (ARP) of AGNR isolates from clinical specimens. It is a base line study to examine the feasibility of routine testing for ESBL production.
Methods: Data over a period of 6 months (April–September 2002) on approximately 100 AGNR isolates from blood cultures, pus and wound specimens and their ARP to the commonly prescribed antibiotics in surgical units were audited. ESBL testing was performed on selected clinical isolates of AGNR resistant to cefotaxime (CTX) or ceftazidime (CAZ) by the NCCLS method of susceptibility testing. ESBL testing was done by the NCCLS method using combination discs.
Results: The overall ARP was co-amoxyclav (58%), cefuroxime (54%), cefotaxime (22%), ciprofloxacin (11%), piptazobactam (9%), gentamicin (6%) and carbapenem (2%). Most of the AGNB isolates resistant to CTX or CAZ were positive for ESBL. Distribution and correlation of data to be presented.
Conclusion: A significant proportion of AGNR show resistance to the most commonly used beta lactam antibiotics in the surgical units. ESBL elaborating AGNB isolates are not uncommon. The ARP may justify routine testing for ESBLs in clinical isolates and use of a carbapenem in an acute clinical scenario when ESBL producing AGNR is the infective agent. Strict hand hygiene and infection control practices should be in place and may play an important role in controlling spread of multiply antibiotic resistant organisms.

P1033 Antimicrobial drug use and imipenem resistance of *Pseudomonas aeruginosa* and *Acinetobacter* spp. strains isolates from nosocomial infections

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Objective: An increase of *Pseudomonas aeruginosa*'s and *Acinetobacter* spp.'s resistance to imipenem was observed during the 1999–2001 period in our hospital. We compared the consumption of antibiotics and the resistance of *P. aeruginosa* and *Acinetobacter* spp. to imipenem in nosocomial isolates of a Greek university hospital during 1999–2001 in order to evaluate the relationship between antibiotic use and resistance prevalence in nosocomial infections.
Methods: AHEPA is 463-bed university hospital with an infectious diseases department that monitors nosocomial infections continuously. Resistance data for imipenem was collected on 1999–2001 about all *P. aeruginosa* and *Acinetobacter* spp. strains isolated from nosocomial infections. Antibiotic consumption was recorded by the pharmacy. The ATC/DDD 2000 methodology was used. The data are expressed as Defined Daily Dose (DDDs) per 100 bed days. There is a restriction policy for third generation cephalosporins, ureidopenicillins and carbapenems in our hospital. Linear regression analysis (SPSS 10) was used to assess relationship between antibiotic use and resistance.
Results: There was not statistical difference in the number of patients or the total bed days during the 3-year period. There was an increase of *P. aeruginosa*'s resistance to imipenem from 4% in year 1999 to 29% in year 2001 and for *Acinetobacter* spp.'s from 1 to 18%, respectively. During the study period there was an increase in the consumption of almost all antibiotics (total antibiotic use DDDs from 47.52 to 50.62, second generation cephalosporins from 10.13 to 12.99, third generation cephalosporins from 3.32 to 3.41 DDDs, ureidopenicillins from 5.47 to 6.42 and carbapenems from 2.73 to 3.36). For *Acinetobacter* spp.'s resistance to imipenem the only correlation found was for carbapenems ($r^2 = 0.9$, $P = 0.01$). For *P. aeruginosa* correlation was found between carbapenems ($r^2 = 0.96$, $P = 0.002$) and second generation cephalosporins ($r^2 = 1$, $P = 0.009$).
Conclusion: Although the total amount of restricted antibiotics is not significantly high, it led to the higher level of *P. aeruginosa*'s and *Acinetobacter*'s resistance to imipenem. The great misuse of second generation cephalosporins should also be monitored, since it affects the resistance trend. Close monitoring of antibiotic use and resistance can have a positive impact on infection control measures.

P1034 Antibiotic prescription rate in hospitalized patients: a multicenter prevalence study

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Accurate information about prescribing patterns in hospitals is valuable in improving the quality of antibiotic prescriptions. Only very limited data on the use of antibiotics in Turkey is available. Data on the use of antimicrobial agents in 18 tertiary care hospitals were collected on March 20, 2002. In this first 1 day point prevalence study the total bed capacities, number of hospitalized patients, the type and number of antibiotic prescriptions, the main diagnosis which the prescription was made, clinical and microbiological evidences for treatment were recorded. One or more antibiotics were ordered in 2900 (30.6%) of 9471 hospitalized patients. The reasons of hospitalization of the patients receiving antibiotics were medical treatment (42.5%), elective surgery (39.6%), treatment of infectious disease (17.1%) and emergent surgical procedures (10.4%). The highest consumption rates were found in surgical (81.6%) and medical (55.2%) intensive care units. The 48.8% of antibiotics were given for treatment and 44.2% for prophylactic use. The most common reasons for treatment were found as lower respiratory tract, urinary tract, surgical wound infections and febril neutropenia. It was impossible to find a reason in 168 (5.8%) patients' records. Antibiotics were ordered empirically in 78.4% of patients. The proven infection rate was found as 30.7%. The 56.4 and 13.4% of orders were evaluated as clinically and microbiologically appropriate, respectively. The most common prescribed antibiotics were cefazolin, ampicillin-sulbactam, ceftriaxone, ciprofloxacin, amikacin, gentamicin, ornidazole, cefuroxime, meropenem and vancomycin. We determined that 67.44% of the patients were in official health insurance systems and 19.7% of them were in official social assistance system. These results suggest that antibiotic prescription and empirical treatment rates were high at inpatient groups.

P1035 Relationships between susceptibility of *Enterobacter* spp. and hospital- and patient-specific variables. Report from the Antimicrobial Resistance Rate Epidemiology Study Team (ARREST Program)

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Introduction: Identification of patients with infection associated with antibiotic-resistant pathogens remains a serious challenge for the study of drug regimens to treat such infections. The ARREST Program was established as a

multidisciplinary, collaborative effort to use surveillance data and analytic techniques to better understand factors associated with antimicrobial resistance. The analyzes presented herein were conducted to identify factors predictive of decreased susceptibility of *Enterobacter* spp. in hospitalized patients.

Methods: 5 years (1997–2001) of North American SENTRY Program data were analyzed. MICs for cefepime (CPM), ciprofloxacin (CIP) and piperacillin/tazobactam (P/T) vs. patient-specific (e.g. age, duration of hospital stay prior to isolate collection (hospital duration), infection source, infection risk factors) and hospital-specific (e.g. bed count, geographical region, study year) variables were analyzed using multivariable general linear modeling (GLM) for censored data with backwards stepwise elimination (at $P > 0.1$).

Results: MIC_{50}, MIC range and percentage nonsusceptible for isolates ($n = 356$, 96% blood, from 30 hospitals) were $\leq 0.12, < = 0.12$ to >16, 0.6 for CPM; $\leq 0.25, < = 0.015$ to > 2, 4.8 for CIP; and 2, ≤ 0.5 to > 64, 22 for P/T. Highly significant variables identified from the GLM models included bed count ($P \leq 0.001$) and hospital duration ($P \leq 0.008$). The proportion of explained MIC variability ranged from 20 to 33%. This range increased to 33–43% when hospital was included as a variable in these models. Higher predicted MICs resulted from combinations of these and other significant variables in the models. Observed MIC_{50} (% non-susceptible) for each agent was compared in selected patient cohorts possessing combinations of variables identified from these models (see Table 1).

Table 1

	Observed MIC_{50} (% non-susceptible)		
Patient cohorts	CPM	CIP	P/T
All patients	≤0.12 (0.6)	≤0.25 (4.8)	2 (22)
Bed count <400	≤0.12 (0)	≤0.25 (2.6)	4 (29)
Bed count <400 and age 41–60 years	0.25 (0)	≤0.25 (11)	4 (28)
Bed count <400 and hospital duration 11–20 or >30 days	1 (0)	≤0.25 (19)	64 (56)
Age 41–60 years and hospital duration 11–20 or >30 days	0.5 (0)	≤0.25 (6.7)	64 (67)
Bed count <400 and hospital duration 11–20 or >30 days and age 41–60 years	1 (0)	≤0.25 (25)	64 (75)

Conclusions: This approach may be used to predict factors associated with decreased susceptibility. Though GLM models explained a moderate proportion of MIC variability, the higher observed MIC_{50} values among certain patient cohorts compared with the entire population were clinically relevant. Increased variability in MIC may be further explained by additional factors (including antibiotic use). Collection of these additional data remain an on-going focus of the ARREST Program. Irrespective of this limitation, it appears that in patient cohorts at risk for infection with less susceptible *Enterobacter* spp., CPM was more active than CIP or P/T.

P1036 Relationships between susceptibility of *Streptococcus pneumoniae* and hospital- and patient-specific variables: Report from the Antimicrobial Resistance Rate Epidemiology Study Team (ARREST Program)

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Introduction: *S. pneumoniae* remains a leading cause of morbidity and mortality worldwide. The ARREST Program was established as a multidisciplinary, collaborative effort to use surveillance data and analytic techniques to better understand factors associated with antimicrobial resistance. The analyses presented herein were conducted to identify factors predictive of decreased susceptibility of pneumococci in hospitalized patients.

Methods: 5 years (1997–2001) of North American SENTRY Program data were analyzed. MICs for amoxicillin-clavulanate (A-C), azithromycin (AZM), cefepime (CPM), ceftazidime (CTZ), ceftriaxone (CTX), clarithromycin (CLAR), erythromycin (ERY), and levofloxacin (LEV) vs. patient-specific variables (e.g. age, specimen type) and hospital-specific variables (e.g. bed count, geographical region, study year) were analyzed using multivariable general linear modeling (GLM) for censored data with backwards stepwise elimination (at $P > 0.1$), yielding one model for each of these eight agents.

Results: Of the 483 blood isolates from 29 hospitals, a range of 41–100% of MIC values were available for individual agents. Significant and frequently identified factors included geographical region (6/8 models) and age (4/8 models). High predicted MICs resulted from combinations of these and other variables identified. Based on the model for CPM, factors predictive of high MICs were the following: geographical region = South-west or South-east, age ≤18 years, and specimen type = lower respiratory. The observed percentage non-susceptible (NS) and MIC_{90} are compared for all agents for cohorts of patients with 2–3 vs. those patients with 0–1 of these variables (see Table 1). The percentage NS was at least three times higher for patients with 2–3 vs. 0–1 variables for 6/8 models (Table 1).

Table 1

	Observed % non-susceptible (MIC_{90})							
Patient cohorts	CPM	A-C	AZM	CTZ	CTX	CLAR	ERY	LEV
All patients	1.9 (0.5)	3.7 (2.0)	11 (1.0)	19 (8.0)	3.5 (0.5)	9.9 (≤0.25)	13 (2.0)	0.5 (1.0)
2 or 3 variables	9.8 (1.0)	19 (4.0)	26 (4.0)	50 (16)	13 (2.0)	26 (2.0)	36 (4.0)	0 (2.0)
0 or 1 variables	1.1 (0.5)	2.3 (2.0)	9.5 (≤0.12)	15 (4.0)	2.3 (0.5)	8.7 (≤0.25)	11 (1.0)	0.6 (1.0)

Conclusions: This approach may be used to predict factors associated with decreased susceptibility. GLM models explained a moderate proportion of MIC variability and the higher observed percentage NS among certain patient cohorts compared with the entire population of isolates is clinically relevant. Further explanation of the variability in MIC will require identification of additional variables (including antibiotic use). Collection of these additional data remains an on-going focus of the ARREST Program. Finally, in patients with higher observed MICs, CPM, CTX and LEV, in contrast to other agents studied, may be more appropriate empiric choices when pneumococci is suspected.

P1037 Relationships between susceptibility of *Pseudomonas aeruginosa* and hospital- and patient-specific variables: Report from the Antimicrobial Resistance Rate Epidemiology Study Team (ARREST Program)

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Introduction: Identification of patients with infection associated with antibiotic-resistant pathogens remains a serious challenge for the study of drug regimens to treat such infections. The ARREST Program was established as a multidisciplinary, collaborative effort to use surveillance data and analytic techniques to better understand factors associated with antimicrobial resistance. The analyzes presented herein were conducted to identify factors predictive of decreased susceptibility of *Pseudomonas aeruginosa* in hospitalized patients.

Methods: Five years (1997–2001) of North American SENTRY Program data were analyzed. MIC for cefepime (CPM), ciprofloxacin (CIP) and piperacillin/tazobactam (P/T) vs. patient-specific (e.g. age, hospital stay prior to isolate collection (hospital duration), infection source, specimen, primary diagnosis) and hospital-specific (e.g. bed count, geographical region, study year) variables were analyzed using multivariable general linear modeling (GLM) for censored data with backwards stepwise elimination (at $P > 0.1$).

Results: MIC_{50}, MIC range and percentage resistant (R) for isolates ($n = 487$, 93% blood, from 33 hospitals) were: 2, 0.5 to >16, 5.3 for CPM; ≤0.25, ≤0.25 to >2, 12 for CIP; and 4, ≤0.5 to >64, 11 for P/T. Highly significant variables and interactions between variables identified from GLM models included hospital duration ($P = 0.008$) and specimen ($P = 0.003$) for CPM; specimen ($P < 0.0001$) for CIP; and hospital duration primary diagnosis

Table 1

	Observed MIC_{50} (% resistant)		
Patient cohorts	CPM	CIP	P/T
All patients	2 (5.3)	≤0.25 (12)	4 (11)
Hospital duration >5 days and primary diagnosis = immunocompromized	4 (8.2)	≤0.25 (10)	8 (20)
Hospital duration >10 days and infection source = lower respiratory	2 (4.6)	≤0.25 (9.1)	4 (32)

($P \leq 0.008$) for P/T, with higher MICs resulting from combinations of these and other variables. Observed MIC_{50} (%*R*) were compared in selected patient cohorts with combinations of variables identified from these models (see table). Among the two patient cohorts shown, MIC_{50} remained stable for each agent while percentage *R* increased markedly for P/T (Table 1).
Conclusions: Data such as these may be used to predict variables likely for decreased MICs. Though GLM models explained a moderate proportion of MIC variability, the higher observed percentage R among certain patient cohorts compared with the entire population was clinically relevant. Increased variability in MIC may be further explained by additional factors (i.e. antibiotic use). Collection of these additional data remains an on-going focus of the ARREST Program. Irrespective of this limitation, it appears that in patient cohorts at risk for infection with less susceptible *P. aeruginosa*, CPM and CIP were more active than P/T.

Epidemiology of resistance 3

P1038 Intra-abdominal infections: a 2-year analysis from the MYSTIC program in Italy (2001–2002)

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Objective: The MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) Program is a longitudinal antimicrobial surveillance study that collects isolates from patients in intensive care units, neutropenia units, cystic fibrosis and general wards (intra-abdominal and lower respiratory tract infections), where meropenem (MEM) is prescribed. Here are reported 2001 and 2002 data from isolated collected in intra-abdominal infections.
Methods: Organisms were tested using National Committee for Clinical Laboratory Standards (NCCLS) methodology. Minimum inhibitory concentration (MIC) values and percentage susceptibilities at NCCLS breakpoints (%S) were obtained for MEM and a range of comparators including: imipenem (IPM), ceftazidime (CAZ), piperacillin + tazobactam (TAZ), ciprofloxacin (CIP), gentamicin (GM), and tobramicin (TOB).
Results: A total of (101 and 96 Gram-negative and 44 and 27 Gram-positive isolates were collected between 2001 and 2002, respectively. *E. coli* (26.2 and 38.2%) was the most common isolate in both year, followed by *K. pneumoniae* (9.6 and 17.1%), *P. aeruginosa* (7.6 and 13.0%) and *S. aureus* (9.6 and 10.6%). In general, the carbapenems (MEM and IPM) were the most active antimicrobial agents tested against the common organisms. The percentages of MEM was the same as or higher than IPM against every organism tested. Like MEM, TAZ also showed good in vitro activity against *P. aeruginosa* whereas lower was the activity of the other antibiotics. All the antibiotics tested demonstrated a high potency against *E. coli*, with the exception of CIP in both years of study (92.1 and 85.1%, respectively). *K. pneumoniae* was 100% susceptible to MEM and IPM throughout the study period while the activity of the other agents was considerably lower (CAZ 78.6 and 80.9%, TAZ 85.7 and 80.9%, CIP and 78.6 and 76.2%).
Conclusion: The carbapenems, in particular meropenem, remain the most active antimicrobials against the range of Gram-negative and Gram-positive bacteria isolated from intra-abdominal infections in Italy. There was no apparent increase in resistance to meropenem over the 2-year surveillance period.

P1039 Antimicrobial usage and resistance trend comparisons from the MYSTIC program in North America (1999–2001)

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Objectives: The MYSTIC Program is a global, longitudinal antimicrobial surveillance network of hospitals or specific units that utilize carbapenems. A defining aspect of the program is the ability to capture antimicrobial consumption data from participating institutions to evaluate usage patterns, individually or in aggregate, compared with emerging resistance. This report evaluates these relationships for enteric Gram-negative bacilli (EGNB) and *P. aeruginosa* (PSA) over the initial 3-year period.
Methods: Between 10 and 16 medical centers participated during 1999–2001, each submitting up to 200 isolates/year (>7000 overall). Antimicrobial usage for each year plus hospital bed capacity, occupancy rates and number of ICU beds were obtained. Usage was evaluated for several parenteral agents including: ceftazidime, cefepime, imipenem, meropenem, gentamicin, ciprofloxacin, and beta-lactamase inhibitor combinations. The parameter of drug usage was defined daily dose (DDD)/100 patient days calculated from total grams administered (WHO definitions). NCCLS susceptibility testing methods and interpretations were followed to assess resistance.
Results: Resistance among EGNB did not significantly change for beta-lactams, but tended to increase for gentamicin (+1.2%) and ciprofloxacin (+3.1%). PSA resistance rates for gentamicin (+9.0%) and ciprofloxacin (+10.2%) increased in contrast to significantly decreased resistance rates for carbapenems (−8.4%). PSA cross-resistance analysis noted direct correlations of ciprofloxacin resistance and resistance to all other classes, lowest degree of correlation was with meropenem. Formulary use changes were noted: increased meropenem and ciprofloxacin use and decreased imipenem, aminoglycosides and cephalosporin use. Changes in ciprofloxacin DDD/100 rates (+4.9) were directly related to EGNB and PSA resistance, while among PSA, usage and resistance were inversely correlated for meropenem (+0.5 DDD/100; −7.7% resistant) and gentamicin (−3.8 DDD/100; +9.0% resistant).
Conclusions: Important trends were identified: (i) relationship between increased ciprofloxacin use and higher resistance among EGNB and PSA; (ii) correlation between ciprofloxacin resistance and resistance to other classes in PSA; and (iii) an inverse relationship between increased use of carbapenems (meropenem > imipenem) and resistance. This illustrates the value of linking usage data to resistance rates as part of global, longitudinal surveillance programs.

P1040 Comparative antimicrobial potency and spectrum of activity for meropenem and nine comparator agents: report from the United States MYSTIC program surveillance study in 2002

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Objective: To monitor meropenem (MEM) activity in 15 US medical centers actively prescribing MEM through the MYSTIC program, a longitudinal resistance surveillance study.
Methods: In 2002, the centers submitted 3047 isolates from the following: *Citrobacter* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *P. mirabilis*, *Serratia* spp., *S. aureus*, coagulase-negative staphylococci, enterococci, *Streptococcus* spp., *P. aeruginosa* and *Acinetobacter* spp. The antimicrobial activity of MEM, imipenem (IPM), ceftriaxone (CTX), ceftazidime (CAZ), cefepime (CPE), aztreonam (AZT), piperacillin/tazobactam (TAZ), gentamicin (GM), tobramycin (TOB), and ciprofloxacin (CIP) was assessed. NCCLS reference broth microdilution methods and interpretative criteria were applied. Extended spectrum beta-lactamase phenotypes were confirmed (clavulanate inhibition) for isolates, with CTX or CAZ or AZT MICs at ≥2 mg/L.
Results: The MEM MIC90 values were 0.03 mg/L for *E. coli* and *Klebsiella* spp., 0.06 mg/L for *Citrobacter* spp., *P. mirabilis*, and *Serratia* spp., and 0.12 mg/L for *Enterobacter* spp. These potencies were 4–32-fold greater than for IPM. MEM demonstrated the highest susceptibility (S) and lowest resistance (R) rates against Enterobacteriaceae (99.8% S, 0.1% R) = IPM (99.8, 0.2) > CPE (99.5, 0.4). Against *P. aeruginosa*, the rank order based on S, R was MEM (93.1%, 4.4%) > TOB (92.2, 6.9) > TAZ (91.5, 8.4). IPM was slightly more active than MEM (MIC90, 16 vs. 32 mg/L) against *Acinetobacter* spp. CIP showed highest R (11.0%, range 2.0–40.6%) followed by aminoglycosides (AMG; 5.2–6.5%) for all Gram-negative isolates. Among methicillin-S-staphylococci, only CIP (6.1–15.6% R) and AMG (1.3–5.5%) showed significant R. MEM, IPM, CTX, and CPE inhibited ≥94.5% of *Streptococcus* spp. (88% of tested strains were pneumococci and beta-hemolytic streptococci).
Conclusions: As reported for previous years (1999–2001), the MYSTIC Program recorded no significant decline in MEM activity, the widest spectrum agent tested. In contrast, CIP and aminoglycoside resistance was elevated and apparently increasing. Continued R surveillance is warranted in these institutions with high broad-spectrum antimicrobial use, where possible continued with quantitative drug utilization statistics.

P1041 No evidence of decreasing susceptibility of *Proteus mirabilis* to meropenem despite >8 years' licensed usage: comparison of recent susceptibility data from the MYSTIC program (1997–2002) with data from the preregistration period (1989–1993)

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Objectives: Species such as *Proteus mirabilis* have recently emerged as therapeutic problems due to mutations that compromise quinolone, cephalosporin and aminoglycoside use. Carbapenem susceptibility data for *P. mirabilis* isolates have been monitored as part of the ongoing Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program. MYSTIC is a global, longitudinal surveillance program providing in vitro activity data for major nosocomial bacterial pathogens in centres using meropenem (MEM) including intensive care units, cystic fibrosis units, neutropenia wards and general wards. To ascertain the effect of MEM usage on susceptibility of *P. mirabilis*, in vitro activity data from the MYSTIC Program to date (1997–2002) were compared with those obtained during the MEM clinical trial program prior to registration of MEM (1989–93).
Methods: MICs (mg/L) of MEM and imipenem (IPM) against 4110 *P. mirabilis* isolates (1507 isolates collected from MYSTIC centers in Europe, N. America, S. America and Asia from 1997 to 2002; 2603 isolates collected from Europe, N. America and S. America from 1989 to 1993) were determined using the agar dilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Percentage susceptibilities (%S) were interpreted using the NCCLS 2001 susceptibility breakpoint of 4 mg/L for MEM and IPM.
Results: The susceptibility (%S) of MEM remained consistently high throughout the preregistration period and the duration of the MYSTIC study: 99.9% during 1989–93 and 99.4% during 1997–2002. However, there was a decrease in IPM percentageS from 98.9% for 1989–1993 to 92.8% for 1997–2002. MIC90 values for MEM and IPM were 0.25 and 2 mg/L, respectively, for 1989–1993 and 0.5 and 4 mg/L, respectively, for 1997–2002.
Conclusions: Comparison of MEM pre- and postregistration susceptibility data found no decrease in susceptibility of MEM despite increased usage of this agent. In contrast, an overall decrease in susceptibility of *P. mirabilis* to IPM over the same time period was noted. Continued surveillance of all classes is warranted to monitor future trends in resistance and focus effective treatment regimens (such as MEM) for infections caused by *P. mirabilis* (Fig. 1).

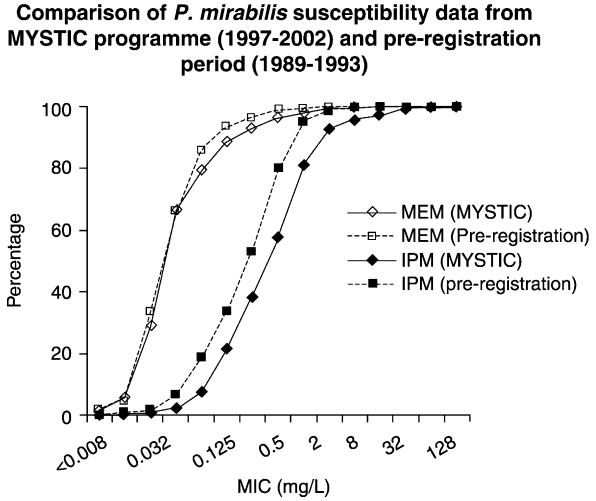

Figure 1

P1042 Worldwide trends in ESBL producing *Klebsiella pneumoniae* – results from the MYSTIC program 1997–2001

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Objectives: Extended spectrum beta-lactamase (ESBL)-producing bacteria are of increasing concern and represent a major challenge to future antimicrobial therapy. ESBLs are most prevalent in *Klebsiella* spp., especially in intensive care and other specialized units. The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program is a global, longitudinal surveillance study monitoring resistance trends in units where meropenem (MEM) is used. The objective of this analysis of MYSTIC data was to determine the incidence of ESBL producing *Klebsiella pneumoniae* and regional trends in frequency and resistance.
Methods: *K. pneumoniae* isolates were collected from MYSTIC Program centres in Europe, N. America, S. America and Asia. Isolates were tested using standard NCCLS methodology against MEM, imipenem (IPM), ceftazidime, piperacillin + tazobactam, gentamicin, and ciprofloxacin.
Results: A total of 2677 isolates were tested overall which included *K. pneumoniae* from Europe ($n = 1688$), S. America ($n = 276$) Asia ($n = 103$), and N. America ($n = 610$). The carbapenems demonstrated the highest activity with MEM ≥IPM in every region except for N. America (99.1% vs. 99.3%, respectively). All the antimicrobial agents tested were more active against *K. pneumoniae* isolates from N. America than from other regions. The percentage of *K. pneumoniae* producing ESBLs was higher in Asia (32.0%) > Europe (30.3%) > S. America (27.2%), and the incidence in all of these regions was considerably higher than that identified in N. America (7.1%). With the exception of the carbapenems, the activity of all antimicrobial agents tested was reduced in ESBL producers compared with non-ESBL-producing isolates.
Conclusion: The carbapenems remain the agent of choice for the treatment of serious infections caused by ESBL-producing *K. pneumoniae*.

P1043 Worldwide susceptibility patterns to *Enterobacter cloacae* from the MYSTIC Surveillance Program 1997–2001

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Objectives: To monitor the susceptibility patterns of *E. cloacae* to commonly prescribed antimicrobial agents as part of the ongoing multinational MYSTIC Surveillance Program.
Methods: In vitro activities of meropenem (MEM), imipenem (IPM), ceftazidime (CAZ), piperacillin/tazobactam (TAZ), ciprofloxacin (CIP) and gentamicin (GM) against 1916 *E. cloacae* isolates (N. America, $n = 330$; S. America, $n = 163$; Europe, $n = 1328$; Asia, $n = 95$) collected from 1997 to 2001 were analyzed and interpreted according to NCCLS 2002 criteria.
Results: Overall, the carbapenems were the most active agents, inhibiting all isolates from S. America and Asia. Moreover, MEM and IPM inhibited ≥99.4% and ≥98.8%, respectively, of isolates from N. America and Europe. Some degree of resistance to IPM ($n = 14$) and MEM ($n = 6$) was reported in a few N. American and European isolates. Isolates from N. America demonstrated higher susceptibility than those from other regions to CAZ (77.3% vs. 62.1–69.3%) and TAZ (81.5% vs. 65.3–76.1%). Susceptibility to GM (96.3%) was much higher in N. American isolates compared with those from S. America (68.7%), Asia (73.7%) or Europe (88.7%). Asian strains were 100% susceptible to CIP, while susceptibility in other regions was <= 93.6%. A susceptibility pattern characteristic of AmpC hyperproducers was found in 29.6, 21.0, 14.0 and 3.9% of isolates from Europe, Asia, S. America and N. America, respectively. Coexistence of other mechanisms of resistance (e.g. extended-spectrum beta-lactamases) was found in a total of 38 AmpC hyperproducers (27 European, 3 Asian, 8 S. American isolates).
Conclusions: Carbapenems remain the most active antimicrobial agents against *E. cloacae*. Continued surveillance is warranted to monitor future resistance trends and guide empiric therapy.

P1044 Lower-respiratory tract infections: a 2-year analysis from the MYSTIC program in Italy (2001–2002)

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Objective: The MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) Program is a longitudinal antimicrobial surveillance study that collects isolates from patients in intensive care units, neutropenia units, cystic fibrosis and general wards (intra-abdominal and lower respiratory tract infections), where meropenem (MEM) is prescribed. Here are reported 2001 and 2002 data from isolated collected from lower respiratory tract infections.
Methods: Organisms were tested using National Committee for Clinical Laboratory Standards (NCCLS) methodology. Minimum inhibitory

concentration (MIC) values and percentage susceptibilities at NCCLS breakpoints (%S) were obtained for MEM and a range of comparators including: imipenem (IPM), ceftazidime (CAZ), piperacillin + tazobactam (TAZ), ciprofloxacin (CIP), gentamicin (GM), and tobramicin (TOB).
Results: A total of 39 and 48 Gram-negative and 16 and 29 Gram-positive isolates were collected between 2001 and 2002, respectively. *P. aeruginosa* (16.4 and 23.4%) was the most isolated microorganism followed by *S. aureus* (10.9 and 24.7%) and *K. pneumoniae* (10.9 and 20.8%). In general, the carbapenems (MEM and IPM) were the most active antimicrobial agents tested against the common organisms. The percentages of MEM was the same as or higher than IPM against every organism tested. MEM showed excellent in vitro activity against *P. aeruginosa*. *K. pneumoniae* was 100% susceptible to MEM and IPM throughout the study period while the activity of the other agents was considerably lower in both years of study (CAZ and 83.3 and 68.8%, TAZ and 83.3 and 68.8%, CIP 83.3 and 75.0%).
Conclusion: The carbapenems, in particular meropenem, remain the most active antimicrobials against the range of Gram-negative and Gram-positive bacteria isolated from lower respiratory tract infections in Italy. There was no apparent increase in resistance to meropenem over the 2 year surveillance period.

P1045 Emergence of multiresistant Enterobacteriaceae in Eastern Romania

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Objectives: Clarification of the mechanisms of resistance of oxyiminocephalosporin and cefoxitin resistant Enterobacteriaceae.
Methods: Strains of *E. coli* ($n = 2$) and *K. pneumoniae* ($n = 6$) isolated from patients of the Galati County Hospital, Galati, Romania between 2000 and 2001 with positive ESBL test were investigated. MICs were determined by an agar dilution technique according to NCCLS guidelines. Analysis of transferrability of plasmids and isoelectric focusing of crude homogenates were performed by standard procedures. The type of beta-lactamase was identified by PCR using beta-lactamase group specific oligonucleotides. The amplified beta-lactamase genes were sequenced.
Results: The strains were resistant to oxyiminocephalosporins, aztreonam and cefoxitin. Beta-lactam resistance was transmissible except resistance to cefoxitin. The pIs were either 8.2 or above 9.0 indicating SHV- and CTX-M-type enzymes. PCRs with SHV- and CTX-M-group specific primers were run. By comparison of the amino acid sequence of the amplified beta-lactamase genes with published sequences the enzymes were identified as SHV-12 and CTX-M-15. Resistance to cefoxitin was not reduced by Ro48–1220, an AmpC beta-lactamase inhibitor, suggesting a nonenzymatic mechanism.
Conclusion: In Moldavia region a rather unique combination of resistance mechanisms namely of CTX-M-15 or SHV-12 production plus reduced permeability evolved among Enterobacteriaceae conferring resistance to all beta-lactams except carbapenems.

P1046 Emergence of metallo beta-lactamase-mediated resistance to Imipenem in enterobacterial isolates in Australia

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A multiresistant strain of *Escherichia coli* and another of *Klebsiella pneumoniae* were isolated from two patients with urinary tract infections in a hospital in Melbourne, Australia. The *E. coli* isolate was resistant to all beta-lactam antibiotics except imipenem to which it showed reduced susceptibility and no synergy was found with the double-disk synergy test using any beta-lactam and clavulanic acid. The *K. pneumoniae* isolate was resistant to all beta-lactam antibiotics including imipenem and synergy was found only between aztreonam and clavulanic acid with the double-disk synergy test. Besides the expanded-spectrum beta-lactamase (ESBL) demonstrated by the double-disk synergy test in the *K. pneumoniae* isolate, preliminary hydrolysis experiments with crude culture extracts revealed that both isolates had carbapenem-hydrolyzing beta-lactamase activity. PCR experiments with whole-cell DNA of each isolate as template and specific primers for blaIMP and blaVIM revealed that these strains had a blaIMP-like beta-lactamase gene. Sequencing identified metallo-enzyme IMP-4 previously found in *Citrobacter youngae* and *Acinetobacter baumannii* isolates from Main China. This beta-lactamase gene was part of a class 1 integron. Conjugation experiments identified a similar plasmid carrying the blaIMP-4 gene in the two isolates. This is the first report of acquired class B metallo-beta-lactamase in Australia. Additionally, this work underlines that these expanded-spectrum beta-lactamase genes may spread not only in *Pseudomonas* spp. but also to the Enterobacteriaceae.

P1047 VIM-1 or VIM-1 + VIM-2 metallo-lactamase-producing *Pseudomonas aeruginosa* isolated from hospitalized children in Poland (1997–2002)

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Objectives: To screen imipenem-resistant *P. aeruginosa* clinical isolates for the presence of metallo-beta-lactamases (MBL) and to characterize MBL-producing strains.
Methods: Minimum inhibitory concentrations (MICs) were determined using the NCCLS agar dilution method. Phenotypic screening involved the MBL Etest strip (AB BIODISK, Solna, Sweden). Biochemical analysis used crude cell extracts examining imipenem (IMP) hydrolysis preincubated with EDTA. Genotyping to determine epidemiologic relatedness was done using pulsed-field gel electrophoresis (PFGE). PCR analysis was undertaken using oligonucleotides based on the conserved regions of imp and vim DNA sequences. Sequencing was determined using DuPont Automated systems and analyzed using DNAstar.
Results: In the period 1998–2002 12 *P. aeruginosa* isolates producing MBL were detected. The Etest produced positive results: IMP MIC at >256 mg/L and IMP/EDTA at 1–1.5 mg/L. Isolates were resistant to aminoglycosides, all beta-lactams (except of aztreonam), and susceptible to ciprofloxacin. PFGE pattern indicated that they were clonally related. Crude extracts showed hydrolysis of IMP, which was inhibited (over 90%) on addition of EDTA. PCR analysis of genomic DNA, in contrast to plasmid DNA, gave a positive result for the presence of vim MBL gene. Sequencing of these products indicated a 100% match with the vim-1 MBL in all tested strains, and seven additionally contained a vim-2 MBL.
Conclusions: This is the first report in Europe concerning isolation of multiple MBL-producing *P. aeruginosa* strains from one hospital in a 5-year period. Seven of 12 isolates produced simultaneously a VIM-1 and VIM-2 MBL.

P1048 Drug resistance pattern of *Pseudomonas aeruginosa* strains isolated from burn infections in Iran

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Background: One of the most important complications of burned patients is burn infection, particularly infections with *Pseudomonas aeruginosa* that occurs after one week of hospitalization. This organism is highly resistant to the antibacterial agents.
Objective: The aim of this survey was the study of antibacterial resistance and Beta lactamase production of *P. aeruginosa* isolated from burn infections. Therefore, 150 isolates of *Pseudomonas* strains were cultured from burned patients at a burn and accident center in Iran.
Methods: All strains were screened for their susceptibilities to antibiotics such as: ciprofloxazine, amikacin, gentamicine, tobramycine, tetracyclin, carbenicilin, ceftazidime, cefteriaxone and cotrimoxazole using disk diffusion method. Beta lactamase production was checked by iodometric method.
Results: The rate of resistance of the strains to ciprofloxazine was less than the other antibiotics: 119 resistance strains = 80%. The results of the resistance to other antibiotics are as follow: ceftazidime = 96.6%, gentamicin = 93.3%, amikacin = 89.3%, tobramycin and kanamycin = 97.2%, carbenicillin, ceftriaxone and cefotaxime = 98.6%, tetracyclin and cotrimoxazol = 100%. Using the iodometric method, we found that 87 strains out of 127 produced Beta lactamase.
Conclusions: Considering the obtained results and high prevalence of multidrug-resistant strains in the hospital environment, it is obviously necessary to detect and follow up the sources of nosocomial infections. This is particularly important for *P. aeruginosa* infections at Burn and Accident Centers to prevent the spreading of drug resistant strains and its transmission. Using the correct drug susceptibility result, would help the better management of such cases.

P1049 Evolution of the antibiotic resistance in *Acinetobacter baumannii* isolates from a hospital in Northern Spain

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Leioa, E

Objectives: The study of the evolution of the resistance and the detection of class 1 integrons from 1999 to the year 2002 among *Acinetobacter baumannii* clinical isolates.
Methods: Minimum inhibitory concentration to ceftazidime, cefotaxime, imipenem, meropenem, amikacin and gentamicin was determined following the NCCLS protocol. Isolates tested were all *A. baumannii* isolated at the Microbiology Service of Hospital de Sta. Marina (Northern Spain) during the years 1999 and 2002. Detection of class 1 integrons was done by PCR experiments with the primers 5′CS and 3′CS.
Results: The most active antibiotic was amikacin during 1999 but imipenem during 2002. Cefotaxime and gentamicin were the lest active during 1999 and also the same during 2002. The resistance to ceftazidime, imipenem, meropenem and amikacin increased being most important to meropenem (from 60.8% to 76.8%) and amikacin (32.3% to 76.8%). The evolution of the integrons was towards the combination of the different individual bands identified during 1999. The majority of isolates from 2002 bored combinations of the 1200 bp, 760 bp and 550 bp integrons.
Conclusions: The resistance of *A. baumannii* to antibiotics increased from 1999 to 2002, specially to meropenem and amikacin. This could be due to the acquisition of multiple class 1 integrons.

P1050 Detection of the *ermTR* gene among erythromycin-resistant *Streptococcus pneumoniae* strains circulating in Italy

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Objectives: Macrolide-resistance in pneumococci has been ascribed to two different genes: ermB conferring constitutive or inducible cross-resistance to MLSB antibiotics and mefA. Strains harbouring this latter gene are resistant only to 14- and 15-membered ring macrolides. At present, the *ermTR* gene, which is widely diffused in *S. pyogenes*, has been found in only four *S. pneumoniae* strains isolated from pediatric throat swabs in Greece, in two Australian and in one Spanish isolates whose origins have not been described. In this study 336 erythromycin-resistant pneumococci have been characterized for their macrolide-resistant genotype.
Methods: Erythromycin-resistant *S. pneumoniae* (336) isolated from various clinical specimens during 1996–2000 in Italy have been studied. Minimal inhibitory concentrations were determined by the microdilution method as suggested by the NCCLS (2002). Macrolide resistance phenotypes were assessed by the double disk test. The *ermB*, *mefA* and *ermTR* genes were amplified by PCR using specific primers and conditions described by other Authors (Syrogiannopolus *et al*. AAC, 2001).
Results: The great majority of the strains (90.5%) possessed the ermB gene, while all M-type pneumococci (9.2%) carried the mefA determinant. Only one strain (0.3%) originating from a pediatric throat swab belonging to serotype 11 A, the same displayed by the Greek isolates, was characterized by an inducible phenotype. The MICs for erythromycin and clindamycin were 1 and <0.06 mg/L, respectively. PCR studies revealed that this strain harboured the *aermTR* gene.
Conclusions: This is the first study showing the presence of the *ermTR* gene among pneumococci circulating in Italy. The rare occurrence of this genetic element in *S. pneumoniae* is therefore confirmed.

P1051 Beta-lactamases in Enterobacteriaceae from pediatric patients in Iasi, Romania

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Objectives: To determine the mechanism of beta-lactam resistance in Enterobacteriaceae causing pediatric infections and to increase the level of awareness of clinical laboratory about the detection of beta-lactamases.
Material and methods: During January–June 1999, *E. coli* ($n=8$), *Klebsiella pneumoniae* ($n=1$), *Enterobacter cloacae* ($n=1$) isolated from urine and pus in children hospitalized in 'Sf. Maria' Pediatric Hospital from Iasi, Romania, were selected by their resistance phenotype, suggesting a beta-lactamase production. Identification and sensitivity testing was done by Dade Micro-Scan system with NegCombo 20E panel. Isoelectric focusing was done in preformed minigels and Ceftazidime (0. 25 μg/mL) was used for bioassay.
Results: The strains resistant to Ceftazidime (4/10) showed association of pIs: $5.4+7.6$ or $5.4+8.2$ suggesting the production of extended spectrum beta-lactamases (ESBLs). This was confirmed by bioassay method with bacterial growth (due to hydrolysis of CAZ) at pIs of 7.6 and 8.2 indicating ESBLs of the SHV type. The beta- lactamases of the CAZ sensitive strains (6/10) focused at pI of 5.4 which corresponds to the production of TEM-1 beta-lactamase.
Conclusion: Results show the emergence of ESBL (most likely of the SHV type) in strains isolated from infections in children hospitalized in a large clinical pediatric hospital. Association of ESBL plus TEM type beta-lactamases (40% of the isolates) impairs the options for treatment of infections caused by these strains. The routine diagnosis clinical laboratory should detect these enzymes, by simple and cheap methods, as showed above. Part of this work was performed at the Bacterial Genetics Department in Cuernavaca, Morelos, Mexico.

P1052 First detection of *Klebsiella pneumoniae* and *Enterobacter cloacae* clinical isolates producing the VIM-1 metallo-beta-lactamase

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Objectives: Multidrug resistance among clinical isolates of Enterobacteriaceae represents an increasing problem. The recently discovered acquired metallo-beta-lactamases (MBLs) of the VIM-type, mostly found in nonfermenting Gram-negative bacteria, are able to efficiently hydrolyze nearly all beta-lactam antibiotics, including carbapenems, and are encoded by mobile genetic elements, thus representing a challenging threat for beta-lactam-based chemotherapy. To-date, in Europe, production of VIM-type enzymes in Enterobacteriaceae has been reported only in one isolate of *Escherichia coli* from Greece. In this study, we report on the detection of the VIM-1 enzyme in *Klebsiella pneumoniae* and *Enterobacter cloacae* isolates from Italy, and on results concerning the molecular characterization of these isolates.
Methods: Antimicrobial susceptibility testing was performed and interpreted according to the NCCLS criteria. The presence and nature of a metallo-beta-lactamase determinant was determined by molecular methodologies (RFLP-multiplex PCR) and enzymatic assay. The genetic background of the MBL-determinant was investigated by conjugation and Southern blot hybridization experiments.
Results: On June 2002, one *K. pneumoniae* and one *E. cloacae* exhibiting resistance to several beta-lactams and reduced susceptibility to carbapenems were isolated from an inpatient at the ICU of the Ospedale di Circolo of Varese, Italy. Both isolates produced EDTA-inhibitable carbapenemase activity. Results of the RFLP-multiplex PCR for the detection of MBL genes indicated the presence of a blaVIM-1 determinant. In either isolate the MBL determinant was carried by an apparently identical plasmid, that was successfully transferred to *E. coli* by conjugation.
Conclusions: The emergence of MBL determinants carried by conjugative plasmids among clinically relevant species of Enterobacteriaceae, especially *K. pneumoniae*, is particularly alarming and underlines the need of a continuous surveillance program of these worrisome determinants.

P1053 The EOLO Project: an epidemiological study of community-acquired lower respiratory tract infections (LRTI) in Italy (2001–2002)

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Objectives: An epidemiological evaluation of bacterial pathogens responsible for community-acquired lower respiratory tract infections (LRTI) and the corresponding resistance patterns to the commonly used antimicrobial drugs.
Methods: This Italian national survey involved 360 general practitioners divided in groups of 12, each group coordinated by a pneumologist with a reference microbiology center.

Results: A total of 860 patients were recruited of which 449 (92%) diagnosed as acute exacerbation of chronic bronchitis (AECB) and 39 (8%) community acquired pneumonia (CAP). Sputum samples were collected from 727 (88,0%) patients of which 515 (70,8%) had samples valid according to Bartlett criteria and were further analyzed and cultured. These 515 patients (67.4% males and 32.6% females) included in the microbiological analysis had mean age of 69.1 years for males and 65.6 years for females. Etiology of AECB varied from area to area but overall 6.5% of cases had a polymicrobial aetiology and pathogens with the highest incidences were: *S. pneumoniae* (12.9%), *H. influenzae* (19.5%), *H. parainfluenzae* (17.9%), *M. catarrhalis* (9.6%), *S. aureus* (15.2%), followed by Enterobacteria and Gram-negative non-fermenting rods. Resistance rate of *S. pneumoniae* to penicillin was 13% (6.5% high level, 6.5% low-level), whereas the resistance to macrolides was 35.7%. One pneumococcal strain only was reported as resistant to moxifloxacin. Beta-lactamases production in *H. influenzae* was 33% and even higher beta-lactamase production rates were expressed by *H. parainfluenzae* and *M. catarrhalis*. *S. pneumoniae* was 14.8% of all strains isolated in CAP, *H. influenzae* 22.2%, *H. parainfluenzae* 18.5%, and *S. aureus* 29.6%.
Conclusions: This epidemiological survey confirmed the causative role of the main pathogens also in Italy as reported elsewhere regards the etiology of AECB and CAP. The incidence of bacterial resistance observed is in agreement with data from previous Italian studies. Moxifloxacin was one of the most active compounds and showed greater activity than the beta-lactams and macrolides tested.

P1054 Occurrence of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strains in a neonatal intensive care unit, Budapest

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Objectives: The present study was designed to determine the occurrence and the antimicrobial resistance of the extended-spectrum beta-lactamase producing of *Klebsiella pneumoniae* (ESBL-Kp) strains isolated from the Neonatal Intensive Care Unit of 1st Department of Obsetricology and Gynecology, Semmelweis University.
Methods: The strains were identified in the automatic ATB system using trips with biochemical tests. ESBL-producing strains were detected with double disk diffusion test and it was confirmed by ESBL screening (ceftazidime/ceftazidime + clavulanic acid) *E*-test. MICs for different β-lactams and aminoglycosides and ciprofloxacin were determined according to the NCCLS guidelines. BlaTEM and blaSHV and blaCTX-M primers were used to amplify the β-lactamase gene. To assess the relatedness between the ESBL-Kp isolates AP-PCR were performed with ERIC2 primers.
Results: From 2001 April to 2002 April 74% of the *K. pneumoniae* strains produced ESBL. Colonization/infection with the ESBL-Kp isolate was detected in 21 patients. Two patients were infected first with ESBL-Kp, and subsequently with ESBL-producing *Serratia marcescens*. The risk factors for infection with ESBL-Kp were the low maturity (delivery at an average of 30 gestations weeks), the low birth weight (average: 1560 g), the prolonged hospital stay, the mechanical ventilation (100%), and previous broad spectrum antibiotic prophylaxis. Out of the 21 strains eight strains harbored the TEM gene, all had the SHV gene, and two strains had the CTX gene. Based on the AP-PCR 10 different genotypes could be distinguished. The susceptibility profiles (range MICs, mg/L) of the ESBL-Kp isolates were as follows: cefotaxime: 2- >32, cefoperazone: 6- >256, ceftriaxone: 1.5- >32, imipenem: 0.125–4, meropenem: 0.023–0.25, piperacillin/tazobactam: 2- >256, amikacin: 2–48, tobramycin:6–24, netilmicin: 8- >256, ciprofloxacin: 0.047–0.25. All isolates were susceptible to imipenem, meropenem and ciprofloxacin and showed low resistance rate to amikacin (10%). Higher and significant resistance rates of ESBL-Kp was observed to cefotaxime, ceftriaxone, cefoperazone, piperacillin/tazobactam (67%) and netilmicin (67%), tobramycin (71%).
Conclusions: This study strongly suggests that ESBL production of *K. pneumoniae* isolates have been highly disseminated among patients in neonatal intensive care unit. There was a high resistance to antimicrobials, and multiresistance rates were increased in the strains producing ESBLs.

P1055 High prevalence of multiresistant penicillin-intermediate *Streptococcus pneumoniae* due to clonal spread in Germany, winter 2000/01

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Objectives: In Germany, penicillin-nonsusceptible *S. pneumoniae* (PNSP) are relatively uncommon, occurring at a rate of about 7% (~6% intermediate and ~1% resistant). The aims of this surveillance study were to assess the prevalence of PNSP recovered from outpatients with RTIs in the winter season 2000/01 and to examine the clonal relationship between PNSP.
Methods: We performed two multicenter studies comprising a total of 17 laboratories. MICs were determined in a central laboratory with a broth microdilution procedure according to NCCLS. Among the antibiotics tested were penicillin (PEN), several other beta-lactams, erythromycin (ERY), tetracycline (TET) and cotrimoxazole (SXT). Fourty-two PNSP were studied using pbp2b restriction fragment length polymorphism (RFLP) and pulsed-field gel electrophoresis (PFGE).
Results: Of the 595 isolates included, 107 (18%) proved PNSP. For those, the PEN MICs were 0.125–0.5 mg/L. None were resistant to PEN. Among the 107 PNSP 84 (78.5%) were of the same phenotype. The most frequent phenotype (pt I) was cross-resistant to TET (98.8%) and had reduced susceptibility to SXT (100%). Sixteen isolates (15%) were addionally resistant to ERY (pt II). The rate of PNSP varied by region ranging from 0% to >40%. Twenty PNSP of pt I and all 16 PNSP of pt II were genetically characterized. Four PFGE types (types 1–4) and 2 different pbp2b RFLP patterns were found among the 20 isolates of pt I. Seventeen of these isolates belonged to PFGE type 1 representing a unique RFLP pattern. They were detected at 7 centers. All 16 isolates of pt II belonged to the same PFGE type (type 5) and represented a different unique RFLP pattern. These isolates were recovered at 3 centers in Eastern Germany. The MICs of two beta-lactams (cefaclor and cefixime) for PNSP exceeded the PK/PD breakpoints.
Conclusions: The rate of PNSP in this surveillance study was higher than reported in previous studies in Germany. There is evidence that this difference is mainly due to the epidemic spread of two clones. Both clones were nonsusceptible to PEN, SXT, and TET, and one was additionally resistant to ERY. The observed regional differences in the prevalence of PNSP is attributed to the spread of these two clones. Regional resistance rates must not be predicted from overall resistance data in a country. The use of standard antibiotics like TET, SXT, ERY and some oral cephalosporins like cefaclor or cefixime contribute to the selection of multiresistance in *S. pneumoniae.*

P1056 Detection of CTX-M-15 extended-spectrum beta-lactamase (ESBL) in the UK

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Objectives: To investigate for the presence of CTX-M type beta-lactamases in isolates of Enterobacteriaceae collected in a survey of bacteria from hospitalized patients in the British Isles.
Methods: Twenty-six hospitals in the UK and Ireland collected up to 200 consecutive clinically significant isolates from separate in-patients in 2001. Disc testing was performed at the hospital laboratories, and isolates with key resistances, including to third-generation cephalosporins, were sent to ARMRL for MIC determinations by the BSAC agar dilution method. Isolates of Enterobacteriaceae (except *Serratia* spp.) with cefotaxime MICs at least 8-fold higher than ceftazidime MICs were screened for blaCTX-M genes by PCR using universal primers. PCR products were sequenced on both strands to determine CTX-M subtype, and group-specific primers were then used to amplify and sequence the entire gene. CTX-M-positive isolates were compared by pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested DNA.
Results: 122 cephalosporin-resistant Enterobacteriaceae were collected. For 7 of these the cefotaxime MIC was at least 8-fold greater than that of ceftazidime. Three of these isolates yielded amplicons with universal CTX-M primers, as did one isolate highly resistant to both ceftazidime and cefotaxime. These 4 isolates were all *Escherichia coli*, and were isolated from

3 hospitals; the isolates from different hospitals were unrelated by PFGE. Sequencing of one representative from each site revealed a CTX-M-1-group enzyme, identified by further sequencing as the CTX-M-15 variant. CTX-M-15 is one of the CTX-M types with relatively greatest activity against ceftazidime, perhaps explaining why the isolates were clearly resistant to this drug as well as cefotaxime. Nevertheless the high level resistance to ceftazidime of the Newcastle isolate suggests the presence of other mechanisms in addition to CTX-M-15.
Conclusions: CTX-M-15 beta-lactamase has emerged at multiple sites in the UK (Table 1).

Table 1

Strain	Species	CTZ MIC	CTX MIC
London (two isolates)	*E. coli*	32	>256
Newcastle	*E. coli*	>128	>256
Belfast	*E. coli*	32	256

P1057 Group A *Streptococcus*: evaluation of antimicrobial susceptibility

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Objectives: The purpose of this study was to evaluate the antibiotic resistance pattern of Group A *Streptococcus* (GAS) strains isolated from children.
Methods: Between September and December 2002, a total of 120 GAS strains were collected from cultures of throat swabs from pediatric patients affected by tonsillitis. Multiple isolates from the same patient were avoided. Strain identification was confirmed with bacitracin disks and by a latex agglutination assay. The susceptibilities tests (penicillin, erythromycin, rokitamycin, clindamycin, tetracycline, ciprofloxacin, vancomycin) were determined by the disk diffusion method on Mueller–Hinton agar supplemented with 5% sheep blood and the macrolide resistance phenotypes were evaluated using the erythromycin-clindamycin double-disk test.
Results: Our findings are showed below: all Group A Streptococcus strains were susceptible to penicillin and vancomycin; 71 of 120 strains (59.1%) were susceptible to all antibiotics tested; 35 (29.1%) strains were resistant to erythromycin; 13 (10.8%) were resistant to rokitamycin; 18 (15%) were clindamycin-resistant; 17 (14.1%) were tetracycline-resistant; 10 (8.3%) were ciprofloxacin-resistant. The macrolide resistance phenotypes test gave the following results: 52.2% of erythromycin-resistant strains were constitutive phenotype; 39.1% M-resistance phenotype; 8.7% inducible phenotype.
Conclusions: This analysis shows that penicillin is still the most effective antibiotic against GAS probably because of the absence of selectable variants carrying mechanisms of beta-lactam resistance. Regarding the prevalence of macrolide-resistance, erythromycin performance is worse than rokitamycin and unlike data reported in literature we have a larger prevalence of constitutive phenotype while inducible phenotype is only 8.7%. Regarding ciprofloxacin, the resistance-prevalence was 8.3% without any association with macrolide resistance (only one strain was constitutive phenotype). This could be a warning signal for a future increase in resistance considering that children are not usually given fluorquinolones.

P1058 Occurrence and spread of extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* isolates in Curaçao

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Objective: In recent years, the growing incidence of ESBLs, in particular produced by *Klebsiella pneumoniae* isolates in nosocomial infections, also became evident in Curacao. To get insight into the evolution of ESBLs and to identify a possible source for the increase of these multidrug resistant strains on this island, *K. pneumoniae* clinical isolates were genotypically characterized.
Methods: Sixty-five ESBL producing *K. pneumoniae* of patients from the St. Elisabeth Hospital were collected from November 1999 until June 2002. Identification and susceptibility testing were done by Vitek and confirmed by additional double-disc synergy tests. Twelve strains were collected between November 1999 until December 2000. Fourteen isolates were collected during a NICU outbreak in 2001, 13 strains were collected outside the NICU during the same year. Twenty-six strains were collected in the first half year of 2002. Microbial strain delineation by ribotyping with EcoR1 has been performed to study clonal spread of the strains. All strains were analyzed for plasmid-encoded SHV type β-lactamase by SHV-specific PCR and SHV-NheI RFLP. To test the possible transfer of this plasmid to an *E. coli* recipient strain, conjugation experiments were performed by liquid mating. Isoelectric Focusing (IEF) was done to identify the β-lactamase profiles of these multidrug resistant strains.
Results: The results showed that most of the 65 ESBL-producing *K. pneumoniae* strains contain plasmid encoded SHV-type β-lactamases. SHV-type β-lactamase of the NICU isolates could be transfered by conjugation. However, with the majority of non-NICU strains no SHV ESBL transconjugants were observed. This may be explained by poor mobilization of the ESBL carrying plasmid. Most of these strains demonstrated a variety of IEF patterns; 38 of the 51 isolates showed a $pI \leq 8.3$ suggestive of an AmpC-type β-lactamase. Ribotype analysis revealed 22 unique types. However, one type was increasingly present. This was not the same type as observed in the NICU outbreak of 2001.
Conclusions: Our data suggest that in Curaçao there is an ongoing outbreak of ESBL-producing *K. pneumoniae*, only in a small part due to horizontal transfer of plasmid-encoded SHV genes. There is a prevalent clone, but it remains to be seen if strains of this type all harboring the same ESBL genes. Characterization of ESBL genes other than SHV will be studied.

P1059 Macrolide-resistance phenotypes in *S. pyogenes*

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Objectives: The aim of this study was the assessment of macrolide-resistance rate in *S. pyogenes* isolates and the evaluation of their phenotypic characterization of resistance in Lakonia region, Greece.
Methods: A total of 231 strains of *S. pyogenes* were isolated in 2-year period (2000–2001) from symptomatic patients, children (174 strains) and adults (57 strains). Most strains were isolated from throat swab cultures (212 strains) and the rest from other clinical specimens. The strains were identified as *S. pyogenes* using standard procedures: (i) negative catalase test, (ii) Gram stain, (iii) beta-hemolytic pattern, (iv) colony size, (v) bacitracin sensitivity, and (vi) group A latex agglutination. Susceptibility to penicillin–erythromycin–clindamycin was determined by the disk diffusion method (NCCLS 1999). The strains were examined phenotypically for resistance to macrolides and lincosamides by the double disk method using disks of erythromycin (15 μg) and clindamycin (2 μg).
Results: All strains were found to be susceptible to penicillin. The overall resistance rate to erythromycin was 20.8% (48 isolates). Resistance to clindamycin was observed in 15 strains (6.5%) and this resistance was inducible in 14 and constitutive in 1 strain. The M-phenotype (efflux resistance mechanism) was found to be predominant among the erythromycin-resistance strains as it was expressed in 33 strains (69%). Fourteen strains (29%) were assigned to the inducible macrolide, lincosamide, streptogramin B resistance phenotype (iMLSB) and only 1 strain (2%) revealed the constitutive cMLSB phenotype.
Conclusions: *S. pyogenes* remains fully susceptible to penicillin. Due to the significantly high rate of resistance to macrolides, susceptibility tests to macrolides and lincosamides should be performed simultaneously in the clinical microbiology laboratories, particularly in areas with high rates of macrolides resistance. Continuous epidemiological surveillance of the antibiotic resistance of this species to macrolides is also needed. The high prevalence rate of the M phenotype in our region makes clindamycin a good alternative choice in patients allergic to penicillin.

P1060 Uropathogens causing community-acquired urinary tract infections

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Objectives: To assess the frequency and antimicrobial resistance of urinary pathogens isolated from patients with community-acquired urinary tract infections (UTI).

Methods: This was a retrospective study using the results of 1510 urine cultures considered as potentially significant. The urine samples were collected over a 3-year period (1999–2001). The criteria to consider a positive urine culture as significant were: (a) symptoms suggestive for UTI, (b) bacterial growth counts >10000 CFU/mL with pyuria >15 WBC/hpf. The identification of the bacteria to the species level was achieved using the API system (BioMerieux) and the susceptibility tests were performed by the disk diffusion method following the NCCLS (1998) recommendations.
Results: During the study period a total of 1529 isolates were collected. The most common uropathogen was *E. coli* (72.7%) followed in decreasing order by: *Klebsiella* spp. (7.3%), *Proteus* spp. (6.3%), *Enterococcus* spp. (3.2%), *S. saprophyticus* (2.1%), *P. aeruginosa* (2%) and others (6.4%). The resistance rates (%) for *E. coli*, *Klebsiella* spp., and *Proteus* spp. to antimicrobial agents were as follows: ampicillin 34.8/100/46.4 amoxicillin/clavulanate 16.5/23/18.5; cefuroxime 2.3/5.3/12.3; cotrimoxazole 17/28.5/26.8; ciprofloxacin 2.1/1.8/4.1; ceftriaxone 0.4/1.7/0; gentamicin 2/0.9/3. ESBL producers were found among *E. coli* isolates (0.26%) and among *Klebsiella* spp. (1.7%). Ampicillin resistance in *Enterococcus* spp. was detected in 12% of the isolates; ciprofloxacin resistance occurred in 41% and none of the isolates was vancomycin-resistant.
Conclusions: Enterobacteriaceae were the most commonly isolated uropathogens and *E. coli* was the predominant bacterium. In our region multiple drug resistant strains have not occurred in significant rates but we isolated ESBL producers strains. The antimicrobial susceptibility results must taken account by the clinicians for a rational use of antibiotics in order to avoid the emergence and spread of multiresistant strains.

P1061 Antimicrobial Resistance Surveillance in Italy: results of AR-ISS project

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Objectives: To monitor the antimicrobial resistance of bacteria of particular interest and clinically relevant by means of a sentinel surneillance laboratory-based network coordinate by the ISS, the Italian Public Health Institute. Results on the first year of activity are presented.
Methods: Since June 2001, ISS collected data on antibiotic resistance related to strains of *Staphylococcus aureus*, *Enterococcus fecalis/fecium*, *Klebsiella pneumoniae/oxytoca*, *Escherichia coli* isolated from blood and of *Streptococcus pneumoniae* from blood and cerebrospinal fluid. 61 clinical microbiology laboratories collecting samples from 71 hospitals participated, using their routine lab methods, sending data on standard paper forms or electronically (17 labs). The data analysis was performed on antimicrobial susceptibility results provided by labs.
Results: From June 2001 to May 2002, we received a total of 3989 non duplicate isolates including: *S. aureus*: 1858 isolates-100% were susceptible to vancomycin., MRSA = 41.5%; multiresistance in MRSA strains: 78% *S. pneumoniae* 396 isolates-10.8% non susceptible to penicillin (2.8 resistant), 37.6% resistant to erythromycin. *Enterococcus fecalis/fecium*: 742 isolates-1.5% of *E. fecalis* and 19.9% of *E. fecium* resistant to vancomycin (VRE). Multiresistance in VRE strains: 57.1% of *E. fecalis* and 72.9% of *E. fecium*. *Klebsiella pneumoniae/oxytoca*: 743 isolates-15.2% reported as ESBL positive, 8% multiresistant. *E. coli* 534 isolates-44.8% resistant to aminopenicillins, 19.7% non susceptible to ciprofloxacin, 3% multiresistant.
Conclusions: According to previous studies, antimicrobial resistance of *S. aureus* and *S. pneumoniae* seems to be stable. The AR-ISS surveillance provided the first national data about antimicrobial resistance of *E. fecalis/fecium* and Gram negative. The level of vancomycin resistance of *E. fecium* was higher than expected and resulted one of the highest in Europe. Aminopenicillin resistance was high both among *E. coli* and *K. pneumoniae*/oxytoca strains. Resistance to amicoglicosides and cephalosporines in *E. coli* is still low, but high compared with what reported in other European countries. The level of *K. pneumoniae*/oxytoca ESBL producing strains need to be confirmed.

P1062 Effects of clarithromycin on oropharyngeal and nasal flora: a double-blind placebo controlled study

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Objectives: The use of antimicrobial agents is associated with the emergence of resistance to these drugs in almost all human pathogens. Whether this also accounts for clarithromycin, as a relatively new macrolide antibioticum, has not yet been evaluated. Therefore, a prospective, placebo-controlled, randomized, double blind study was performed to investigate the effect of clarithromycin slow-release on colonization and resistance in oropharyngeal and nasal flora.
Methods: 296 patients with documented coronary artery disease were randomized to receive a daily dose of clarithromycin 500 mg slow release (CL) or placebo tablets (PB) until the day of surgery, during the preoperative period. Nose- and throat swabs were taken before initiating therapy and after. Minimal Inhibitory Concentration (MIC) was determined for oral *Hemophilus* species and nasal *Staphylococcus* (S) *aureus*. Throat swabs were also cultured for genotypic macrolides, lincosamides, and streptogramins b (MLS) cross-resistance detection in indigenous flora.
Results: Basic patient characteristics were comparable in the two treatment groups. The average number of used tablets was 15 (SD = 6.4). Oropharyngeal carriage of *Hemophilus* species was not influenced in either of the treatment groups (79.1% before treatment in both groups, and 70.3% after CL vs. 74.3% after PB). Nasal carriage of *S. aureus* however, decreased significantly from 34.7% before treatment, to 4.9% after CL treatment (compared with PB: 32.4% before and 30.1% after; RR = 13.2). The resistance for clarithromycin was significantly increased in *Hemophilus* species ($P = 0.0002$; RR = 1.9; 95% Confidence Interval: 1.4–2.7). The MLS resistant bacteria accounted for 37% of the total indigenous flora. Directly after therapy and 8 weeks after therapy, the percentage of MLS resistant bacteria increased significantly in patients who received clarithromycin (77% in CL group vs. 30% in PL group directly after, and 53% (CL) vs. 27% (PL) 8 weeks after).
Conclusion: These results show a rapid effect of clarithromycin slow-release on resistance, and suggest the capability of transfer of MLS resistance genes from indigenous flora to pathogenic flora.

P1063 *Staphylococcus* spp. resistance to macrolides and lincosamides

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Clindamycin, a derivative of lincomycin, is active against *Staphylococcus* spp. when the microorganism is susceptible to this antimicrobial agent. A significant number of staphylococci though, are susceptible to clindamycin (ClS) and resistant to erythromycin (ErR). The most common mechanism of resistance to macrolides-lincosamides-streptogramins B (MLSB) is the modification of the antimicrobial binding site due to the methylation of 23S rRNA at the 50S ribosomal subunit. The expression of this resistance can be constitutive or inducible and is related to the acquisition of the *erm* gene. This study was performed aiming to the determination of the frequency of the *Staphylococcus* spp. that are ErR/ClS at Hippokrateio General Hospital, Greece. In total, 2364 non-repetitive clinical strains of *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) that were isolated from various pathological samples during the period of 1998 and 2002 were studied. 257 (23.66%) out of the 1086 clinical strains of *S. aureus* were resistant to both antibiotics, while 80 (7.34%) strains were resistant to erythromycin and sensitive to clindamycin. 93.75% of these strains were oxacillin sensitive (MSSA) and only 6.25% were oxacillin resistant. Among CoNS strains, 527/1278 (41.24%) was resistant to erythromycin and clindamycin, while 286 (22.38%) exhibited the ErR/ClS phenotype. Most of this phenotype strains were oxacillin resistant (66.43%) and 33.57% were oxacillin sensitive. According to these results, the ErR/ClS phenotype is observed mainly to MSSA strains in contrast to CoNS where it is most frequently observed to

oxacillin resistant isolates. The presence of inducible resistance to clindamycin was determined by the double disk test in 18 *S. aureus* and 16 CoNS strains. Inducible resistance to clindamycin was observed to 18/18 (100%) of *S. aureus* and 8/16 (50%) CoNS isolates, demonstrating a large number of ErR/ClS Staphylococcus strains possessing the *erm* gene and therefore the emergence of constitutively resistant mutants during treatment is likely to occur. In conclusion, patients infected with staphylococci exhibiting inducible resistance, should not be treated with clindamycin or should be under continuous surveillance because the selection of resistant isolates to all MLSB during therapy is a risk. Furthermore clinical studies are necessary to evaluate in vivo whether clindamycin should be used and when.

P1064 Activity of vancomycin, teicoplanin and linezolid against coagulase-negative staphylococci

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Objectives: To follow the antimicrobial susceptibility of coagulase-negative staphylococci (CoNS), isolated from wounds of patients of the 1st surgical clinic, from wounds and hip prostheses infections of patients of the 1st orthopedic clinic, and from hemocultures of all patients from the Faculty Hospital.
Methods: In the period from 2000 to 2002, staphylococci were isolated from the blood and swabs from deep wounds after cultivation on blood agar, were identified by plasmacoagulase production and biochemical tests. MIC to vancomycin, teicoplanin, linezolid, quinupristin/dalfopristin, ciprofloxacin, clindamycin, gentamicin, and rifampin was determined by colorimetric method. Resistance to methicillin/oxacillin was determined by the disk-diffusion test according to the NCCLS and verified by the PBP2a detection; resistance to vancomycin was confirmed by the *E*-test.
Results: 692 strains of CoNS were tested, of which 504 showed methicillin resistance (72.8%). Highest percentage of methicillin-resistant CoNS was isolated from hemocultures (76.1%) and from wounds of patients of the 1st orthopedic clinic (73.7%). Out of 692 strains, 132 (19.1%) were resistant to gentamicin, and 261 (37.7%) to ciprofloxacin. Co-resistance in the methicillin-resistant strains was found to gentamicin in 121 cases (24.0%), and to ciprofloxacin in 238 cases (47.2%). 15 strains (2.2%), out of 692 strains, showed teicoplanin resistance. Only 8 strains (1.2%) were resistant and 9 strains (1.3%) intermediately susceptible to linezolid. Resistance to this drug did not exceed 1% in methicillin-resistant strains. All 692 investigated CoNS strains were vancomycin susceptible.
Conclusion: In vitro tests showed that vancomycin can be applied in the therapy of all cases of severe wound infections and sepses, caused by coagulase-negative staphylococci. Teicoplanin and linezolid were ineffective in less than 2.2 and 1.2% of the total number of isolated coagulase-negative staphylococci, respectively.

P1065 Targeted recombinant β-lactamase (Trbl) prevents emergence of ampicillin induced resistance in Enterobacteria

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Objectives: Ampicillin induces changes in the gut microflora composition, concentrations and antibiotic resistance patterns of coliforms. The aim of the study was to determine whether targeted recombinant β-lactamase (TRBL) could overcome the adverse effects of ampicillin by degrading intestinal ampicillin with oral, in time and pH controlled form released, TRBL supplementation.
Methods: 18 male laboratory beagles were randomized in 3 treatment groups (ampicillin + placebo, ampicillin + TRBL or only placebo). Stool samples were cultured before (0 days), during (4, 10, 14 days) and 2 weeks after (28 days) the supplementation period. A total of 860 fecal coliform isolates were collected and tested against ampicillin and 8 other antimicrobial agents.
Results: In ampicillin group all dogs became carriers of ampicillin resistant coliforms and the proportion of ampicillin resistant strains of all isolated coliforms increased dramatically (2%→98%) replacing practically all sensitive strains. On contrary, in TRBL group no new dog became a carrier during the intervention and the proportion of ampicillin resistant strains increased only mildly (20%→36%). In addition, the recovery to baseline-like distribution of ampicillin resistance among coliforms after the supplementation period occurred more intensely in TRBL than in ampicillin group. In placebo group the proportion of fecal ampicillin resistant strains increased steadily from 16% on day 0–50% on day 28. The acquisition of the resistant strains in placebo group has most likely happened via ingestion of resistant flora, as the dogs were not totally isolated from each other.
Conclusion: These results indicate that oral β-lactamase administration (TRBL) can prevent the increase of ampicillin resistance among coliforms in gut microbiota.

P1066 Low prevalence of reduced susceptibility to glycopeptide in methicillin-resistant *Staphylococcus aureus* from Belgian hospitals, 2001

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Objectives: To determine the prevalence of methicillin resistant *S. aureus* (MRSA) with reduced susceptibility to glycopeptide in a national survey conducted in 2001 in Belgian hospitals.
Methods: In 2001, 455 MRSA clinical isolates from various body sites were collected from 98 acute-care hospitals. Glycopeptide susceptibility was screened by MIC agar dilution method, vancomycin screen agar (BHI + 6 mg/L) and synergy/antagonism test with aztreonam/cefazolin on Mu3 agar (BHI + 3 mg/L vancomycin) and confirmed by population analysis and broth microdilution.
Results: By dilution, vancomycin MIC_{50} and MIC_{90} were 1 and 2 mg/L, respectively (ranging from 0.25 to 4 mg/L) and teicoplanin MIC_{50} and MIC_{90} were 0.5 and 2 mg/L (ranging from 0.06 to 8 mg/L). Only four isolates grew on vancomycin screen agar (1–2 colonies) but were vancomycin susceptible by the agar dilution method (MIC equal or less than 2 μg/mL). On Mu3 agar, 31 MRSA strains grew with inhibition around cefazolin disk and enhanced growth around aztreonam disk (putative GISA phenotype). Thirty-six strains that either had vancomycin MICs of 4 mg/L ($n = 6$) and/or teicoplanin MICs ranging from 4 to 8 mg/L ($n = 9$) or grew on vancomycin screen and/or Mu3 agar were submitted to population analysis and microdilution broth. Heterogeneous vancomycin intermediate *S. aureus* (hetero-VISA) with equal or more than 1/100 000 subpopulation growth at 4 μg/mL was found in one (0.2%) isolate. By pulsed-field gel electrophoresis, this hetero-VISA isolate belonged to gentamicin-resistant genotype A4 which is increasingly less frequent in Belgium.
Conclusions: No vancomycin intermediate or resistant *S. aureus* were found and hetero-VISA were very infrequently encountered among Belgian MRSA strains in 2001. Mu3 agar screening showed insufficient specificity for GISA detection in this setting.

P1067 A Spanish national surveillance study among uropathogens that cause community-acquired urinary tract infection

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Objectives: To assess the etiology and rates of antimicrobial susceptibility among uropathogens recovered from outpatients with urinary tract infections (UTIs), mainly lower UTIs, in both national and regional settings in Spain.
Methods: A prospective, multicenter study was performed between April and July 2002. Fifteen microbiology laboratories located in 9 regions were enrolled. All urine samples were obtained from outpatients. Culture procedures, biochemical identifications and antimicrobial susceptibility testing were performed by each participant laboratory using their usual standard methods.
Results: A total of 2674 patients were included, 80.3% women and 19.7% men. The mean age was 53.8 years (10.4% <20 years, 20.9% 21–40 years, 21.3% 41–60 years, 35.2% 61–80 years, and 12.2% >81 years). Patients that suffered their first episode of UTI acount for 52.5% and those with uncomplicated UTI accounts for 45.5%. A total of 2724 isolates were recovered: *Escherichia coli* 73%, *Proteus* spp. 7.4%, *Klebsiella* spp. 6.5%, *Pseudomonas aeruginosa* 1.3%, *Enterococcus* spp. 4.8%, *Streptococcus agalactiae* 1.7% and *Staphylococcus saprophyticus* 0.7%. The overall antimicrobial susceptibilities of *E. coli* isolates were as follows: fosfomycin 97.9%, cefexime 95.8%, nitrofurantoin 94.3%, amoxicillin-clavulanate 90.8%, cefuroxime 90.7%, norfloxacin 77.3%, ciprofloxacin 77.2%, TMP-SMZ 66.1%, and ampicillin 41.3%. Resistance of *E. coli* to fluoroquinolones was significant higher in men (28.9% vs. 19% in women), aged patients (7.1% in <20–40 years, 15% in

41–60 years, 29.2% in 61–80 years, and 33.7% in >81 years), complicated UTI (24.8% vs. 13.7% in uncomplicated UTI) and in some regions (9.2% in Galicia and Asturias, 24.8% in Valencia, and 33.3% in Aragon).
Conclusions: In outpatients, *E. coli* accounts for more than two-thirds of the microorganisms causing lower UTIs. Almost all *E. coli* isolates were susceptible to fosfomycin, cefexime and nitrofurantoin, and 90,8% to amoxicillin-clavulanate. Rates of fluoroquinolones resistance are globally near 22%, but vary significantly according with sex, age, type of UTI, and geographic region. This information needs to be considered when empirical therapy has to be recommended or instaured in Spain.

Non-molecular diagnostics: viral, parasites, fungal and clinical

P1068 Intrathoracic lymphadenopathy in adults: a review of 30 cases

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Objectives: Intrathoracic (hilar and/or mediastinal) lymphadenopathy (LAP) is caused by several disorders such as tuberculosis, sarcoidosis, lymphoma, and metastasis. The rate of these disorders vary according to the clinics and geographic areas. In this study, we aimed to determine (i) clinical findings, (ii) radiographic features, (iii) diagnostic methods, (iv) etiologic distribution of the patients with intrathoracic LAP followed during last 10 years (1993–2002).
Methods: For diagnosis, beside compatible clinical and radiographic features, microbiological and/or histopathologic features are used. Broncoscopic (bronchoalveolar lavage and/or transbronchial biopsy) and/or mediastinoscopic methods were used for obtaining clinical samples.
Results: The etiologic distribution was as follows: mediastinal TB lymphadenitis ($n=10$), primary TB (9), sarcoidosis (9), and lymphoma (2). In TB cases, thoracic imaging (chest X-ray and CT) revealed hilar and/or mediastinal LAP in all, and additionally parenhcymal involvement in six of the patients with primary TB. Bilateral hilar LAP (BHL) was seen in eight of sarcoidosis patients, two of primary TB patients and none of the remaining. All of primary TB patients and six of sarcoidosis patients had presented with erythema nodosum (EN), but none of mediastinal LAP and lymphoma had EN. Tuberculin skin test was positive 17 out of 19 (90%) TB patients, but 2 out of 9 sarcoidosis patients.
Conclusion: TB is the leading etiology of intrathoracic LAP in our country where TB is endemic. On the other hand, sarcoidosis should be considered in patients admitted with BHL and EN and with a positive tuberculin skin test.

P1069 Diagnostic laparotomy in the diagnosis of fever of unknown origin

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Objectives: Fever of unknown origin (FUO) was firstly defined in 1961 by Petersdorf and Beeson as following: Fever greater than 38 °C in several times and lasting longer than 3 weeks and diagnosis is obscure after examination for 1 week in the hospital. Detailed medical history, physical examination, noninvasive laboratory tests and radiological examinations constitute the first level in approach of the diagnosis. Excisional biopsy, fine needle biopsy, and finally laparotomy are among the invasive methods that could be applied in diagnosis of cases in whom the above methods are insufficient.
Methods: In this study we aimed to evaluate the place and contribution of laparotomy in the diagnostic approach of FUO. Of 126 cases with FUO followed up in our clinic between January 1984 and March 2001, 17 cases in whom laparotomy was performed were enrolled in this study. The diagnostic rate of laparotomy and etiology of the FUO by this intervention were identified.
Results: The diagnostic rate of laparotomy was found as 88%. Tissue samples were taken from spleen, liver, mesenteric lymph nodes during operation. As a result of pathologic examination of these tissue samples; miliary tuberculosis ($n=4$), non-Hodgkin's lymphoma (3), Hodgkin's lymphoma (3), liver tumor (1), hairy cell leukemia (1), adult-onset Still's disease (2), and peritonitis carcinomatosa (1) were diagnosed. No diagnosis could be reached in two cases.
Conclusion: After rapid evolution of diagnostic methods especially those of imaging modalities, laparotomy is less frequently used. However, it contributes to the diagnosis in a considerable extent. In selected FUO cases, this invasive intervention will identify the etiology of FUO.

P1070 Interphase tensiometry of cerebrospinal fluid in patients with meningitis

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Objectives: It is surveyed 40 patients with maningitis (20 with purulent and 20 with a serous inflammation of meninges).
Materials and methods: Superficial tension (ST) of cerebrospinal fluid (CSF) investigated methods of the maximal pressure in vesicle (Tensiometer MPT2 the firm-manufacturer LAUDA, Germany) and a hanging drop (Tensiometer PAT1, Sintech, Germany) in a time of interval from 0.001 up to 10,000 s. Determined value of ST (σ) at the initial moment of time $t=0(\sigma 1)$, at $t=0.01$ s ($\sigma 2$), $t=100$ s ($\sigma 3$), $t=1200$ s ($\sigma 4$). Expected a tangent of a corner of an inclination of tensiogramm (λ) in coordinates time of life of a surface – ST at $t=0(\lambda 1)$, and at restoration of the initial form of vesicle after his stressful relaxation 2, registering at this time of a relaxation (ô) and calculating module binding elasticity ϵ by that formula:

$$\varepsilon = \frac{A\Delta\sigma}{\Delta A}$$

where A is the area of a surface of a drop, $\Delta\sigma$ growth of ST, caused by spasmodic change of the area of a drop ΔA.
Results: We reveal moderate positive correlation between concentration of electrolytes and ST of CSF (with concentration of sodium $-r=0.42$, $P=0.14$, potassium $-r=0.51$, $P=0.30$ and chlorine $-r=0.68$, $P=0.04$). In process of increase life duration of a surface correlation connection between electrolytes and ST becomes negative (coefficients of correlation between $\sigma 3$, sodium, potassium and ions of chlorine are equal, accordingly $r=0.134$, $P=0.70$, $r=0.41$, $P=0.22$, $r=0.29$, $P=0.38$. On our data, correlation between ST and concentration of glucose in CSF also varies in process of increase of life duration of a surface (coefficients of correlation with σ_1 $r=-0.42$, $P=0.31$; σ_3 $r=0.40$, $P=0.03$). ST of CSF is determined mainly by increase of the correlation between glucose and protein of CSF $r=-0.61$, $P<0001$). The high contents of protein in CSF assumes high values of the module binding elasticity ($r=0.68$, $P<0.05$) and small time of a relaxation of a surface ($r=-0.61$, $P<0.05$). A corner of an inclination λ characterizes size of adsorption and the contents of protein. In this connection the revealed close correlation between the contents of protein $\lambda 1$ ($r=0.42$, $P<0.05$) and $\lambda 2$ ($r=0.76$, $P<0.05$) is represented.
Conclusions: Distinctions in size of ST of CSF in the field of short times of life of a surface are caused by the maintenance in its electrolytes. Concentration of the last is inversely proportional to the contents of protein in liquor. The basic distinctions in size of ST of CSF, irrespective of time of life of a surface, finally are caused by the contents of protein.

P1071 The impact of a sore throat score in streptococcal pharyngitis

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Objective: To establish the validity of a clinical sore throat score (proposed by CDC) in Infectious Disease Hospital from Iasi, Romania. and to find out if this score will reduce the number of antibiotic prescriptions for upper respiratory tract infectious.
Methods: This study included 168 patients hospitalized between January 1 and November 1, 2002 in Infectious Disease Hospital from Iasi, Romania. The sore throat score had five criteria: age (3–15, 15–45, >45), temperature higher than 38 °C, absence of cough, tender anterior cervical nodes, tonsillar swelling or exudate. The score range is −1 to 5. Pharyngeal swabs were

collected from each patient for throat culture (considered gold standard). Culture was done on blood agar, plates were incubated aerobically for 48 h. In this study we included only patients with no antibiotic therapy prior examination and without other infectious diseases.
Results: Of the 168 patients, 53 (31.5%) had a positive culture result for beta hemolitical streptococci. Most of the patients 106 (63%) was between 15 and 44 years of age. Temperature higher than 38 °C was present in 81 (48.2%) patients, absence of cough in 79 (47.1%), tender anterior cervical nodes in 20 (11.9%) and tonsillar swelling or exudate in 125 (74.4%). Antibiotics were prescribed in 158 (94.04%) cases. Compared with throat culture a score of 3 or higher than 3 has a sensitivity of 62.26%, a specificity of 78.26%, a positive predictive value of 56.7% and a negative predictive value of 81.81%. Efficiency of this score is 73.21%.
Conclusions: All the patients with score 0 should be excluded from culture with no antibiotic therapy. Although several other studies extend this conclusion to patients with score 1 in our study from 31 patients with score 1, 4 (2.3%) had a positive culture for beta hemolitical streptococci so they should not be excluded from culture or a rapid antigenic test (if it is available). Using this score the prescription of antibiotics would have been reduced in our study with 28.5%.

P1072 Value of the naproxen test in the differential diagnosis of prolonged febrile illnesses

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Objectives: Several studies in oncological patients have suggested that the antipyretic response to naproxen is sufficiently specific to distinguish tumor-related fever from infectious fever. We sought to determine the sensitivity and specificity of the 'naproxen test' in unselected patients presenting with a prolonged febrile illness of unknown etiology.
Methods: From a prospectively collected cohort of 290 patients admitted to a single university center with a prolonged unexplained feverish illness, we reviewed the charts of a group of 77 patients who received a non-steroidal anti-inflammatory drug (NSAID), mainly naproxen, during the diagnostic work-up. A complete reponse to the NSAID ('positive naproxen test') was defined as a decrease of maximal body temperature to below 37.2 °C. The final diagnosis was established at the time of discharge from the hospital or during follow-up and the response to the NSAID was compared in the different diagnostic categories.
Results: A complete antipyretic response to the NSAID occurred in 6 of 11 (54.5%) patients with neoplastic fever vs. in 25 of 66 (37.9%) patients with nonneoplastic fever ($P = 0.48$). The sensitivity, specificity, and accuracy of the naproxen test for neoplastic fever were 55, 62, and 61%, respectively.
Conclusion: The naproxen test is not specific for tumor-related fever and has no differential diagnostic role in the work-up of a patient with prolonged unexplained fever.

P1073 Routine use of the new commercial test, GLABRATA RTT for the rapid identification of *Candida glabrata* in six laboratories

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GLABRATA RTT (Fumouze Diagnostics, France) is the first commercial test based on the ability of *C. glabrata* to hydrolyze trehalose but not maltose, within 20 min. It uses a glucose-oxidase reaction to detect glucose, and requires only 4–6 colonies.
Objectives: To assess the performance of this test in the real clinical setting of six laboratories from six countries.
Methods: A total of 275 freshly isolated yeasts including 191 *C. glabrata* and 84 non-*C. glabrata* strains were tested. These strains were isolated either on *Candida* ID from BioMerieux (50 strains in Laboratory A), or CHROMagar *Candida* from Becton Dickinson (60 strains either in Lab. B, or in Lab. C) or Albicans ID2 from BioMerieux (45 strains in Lab. D) or Sabouraud 4% dextrose from Biolife (86 strains in Lab. E) or Sabouraud Chloramphenicol 4% Dextrose from BBL (29 strains in Lab. F). All the strains were fully identified using standard methods.
Results: Using *Candida* ID medium (Lab. A), the GLABRATA test showed a sensitivity and a specificity of 100%. Using CHROMagar *Candida* (Lab. B + Lab. C), the sensitivity and specificity were 93.3 and 100%, respectively. Using Albicans ID2 (Lab. D), the sensitivity and specificity were 100 and 92%, respectively. Using Sabouraud Dextrose from Biolife (Lab. E) the sensitivity was 94.2%. Using Sabouraud Chloramphenicol 4% Dextrose from BBL (Lab. F) the sensitivity and specificity of the test were 42.8 and 100%, respectively. Overall, the GLABRATA test allowed the identification of 175 of 191 *C. glabrata* strains (sensitivity 91.6%) and only 2 of 84 isolates of other yeast species yielded a false-positive result (specificity 97.6%). If the data from Lab. F, where 8 of the 14 *C. glabrata* strains gave an uninterpretable result were excluded, the sensitivity increased to 94% and for the five other laboratories sensitivity and specificity were consistent from one laboratory to another. In Lab. E, the 5 *C. glabrata* strains yielding a false-negative result gave a positive result after a second testing. On the three chromogenic media which allow a good discrimination of the colonies, in 93.1% of cases, the test could be performed directly from the primary isolation medium thus reducing the time for the instauration of an efficient treatment.
Conclusion: The rapid and easy to perform GLABRATA test proved to be highly reliable for the identification of *C. glabrata* in routine use of clinical laboratories.

P1074 Fungi isolated from bile of patients undergoing bile duct surgery

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Objectives: The study comprised the patients undergoing surgery in the tertiary care hospital in Warsaw (1200 beds) during 2 years (2000–2001) due to the gallbladder and bile ducts pathology.
Methods: Fungal strains were cultured from the following clinical specimens: bile and swabs from the drains of the bile ducts, taken before and after the operation. The culture was done according to the standard mycological procedures and commercially available tests (BioMerieux, Sanofi Diagnostics Pasteur). Susceptibility to antifungal agents was tested using an *E*-test system (AB BIODISK, Sweden).
Results: In total 62 specimens were cultured (in 2000–2023; in 2001–2039). Out of them 62 strains of yeast-like fungi were isolated. *C. albicans* was the most commonly isolated species – 38 strains (61.3%), followed by *C. glabrata* – 7 strains (11.3%) and *C. inconspicua* – 7 strains (11.3%), *C. tropicalis* – 4 (6.5%), *C. parapsilosis* – 3 (4.8%), *C. krusei* – 1 (1.6%), *C. valida* – 1 (1.6%) and *C. guilliermondi* – 1 (1.6%). *C. albicans* strains were sensitive to amphotericin B and fluconazole. All strains of *C. inconspicua* were susceptible to amphotericin B and itraconazole.
Conclusion: *C. albicans* was the fungal species most frequently isolated from the bile of surgical patients. Strains of *C. inconspicua* were characterized by the highest frequency of resistance to antifungal drugs.

P1075 Evaluation of *Candida* ID 2, a new chromogenic medium for yeasts

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Objective: *Candida* ID 2 chromogenic medium (BioMérieux, France) is an evolution of *Candida* ID for the isolation of yeasts, the identification of *Candida albicans* (blue colonies) and differentiation of other species belonging to the *C. tropicalis*, *C. guilliermondii*, *C. kefyr* and *C. lusitaniae* group (pink colonies). This medium includes a novel patented specific inhibitor of the *C. tropicalis* hexosaminidase enzyme. The other species of yeast give white colonies.
Methods: One hundred and fifty-eight yeast strains (158) and 61 bacterial were isolated on *Albicans* ID 2 (ALB), *Candida* ID (CAND), and *Candida* ID 2 (CAN2) in order to study fertility and selectivity of these media. Growth, size and coloration of the colonies were monitored after 24 h of incubation at 35 °C. Results obtained with collection and clinical strains have shown that selectivity for bacteria is superior on CAN2 especially for strains from clinical specimens. Data showed that 97% of the strains tested were inhibited on CAN2 against 82% on CAND. Growth, size of colonies and sensitivity on CAN2 have been improved; 41% of the strains grow in larger colonies size on CAN2 compared with CAND and ALB. The intensity of coloration with CAN2 has been improved for 83% of *C. albicans* strains allowing a better reliability. No false negative results was seen on CAN2 whereas one strain of *C. albicans* was colorless on CAND and 2 strains on ALB. In addition no false

positive results were given for *C. tropicalis* strains on CAN2. According to these results, detection of mixed cultures should be easier than ever with three perfectly distinct colony colors for this new chromogenic medium.
Conclusion: *Candida* ID 2 shows a significant increase in sensitivity and specificity of identification of *C. albicans*; no *C. albicans* false-negative results were found. It enables the identification of *C. albicans* as well as the discrimination between *Candida glabrata* and *C. krusei* (white colonies) and the *C. tropicalis*, *C. guilliermondii*, *C. kefyr* and *C. lusitaniae* group. Complete identification of the main yeast species in clinical specimens were mainly obtained in 24 h with possible confirmation at 48 h.

P1076 Performance of Chromagar *Candida* and BIGGY Agar for presumptive identification of yeasts

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Objectives: The importance of identifying the pathogen as quickly as possible has encouraged the development of differential media for the identification of yeasts. In this study two differential media, CHROMagar *Candida* (CHROMagar, French) and BIGGY agar (Nickersen agar) (Oxoid, England) were evaluated for the presumptive identification of yeast species.
Methods: A total number of 270 yeast strains including 169 *Candida albicans*, 33 *C. tropicalis*, 24 *C. glabrata*, 18 *C. parapsilosis*, 12 *C. krusei*, 5 *Trichosporon* spp., 4 *C. kefyr*, 2 *C. lusitaniae*, 1 *Saccharomyces cerevisiae* and 1 *Geotrichum candidum* were included. The strains were first identified by germ tube test, morphological characteristics on cornmeal Tween 80 agar and Vitek 32/API 20 C AUX systems. In parallel, they were also streaked onto CHROMagar *Candida* and BIGGY agar plates which were prepared according to the manufacturers' recommendations. The results were read according to the color, morphology of the colonies and the existence of halo around them after 48 and 72 h of incubation at 370 °C in the dark.
Results: All the strains did grow and the properties of the colonies were clearly distinguished after 48 h of incubation. The sensitivity and specificity values for *C. albicans* strains were found to be 99.4, 100% for CHROMagar *Candida* and 87.0, 75.2% for BIGGY agar, respectively. The sensitivity values for CHROMagar *Candida* changed between 90.9 and 100% while the specificity rate was 100% for other *Candida* species. The sensitivity rates for BIGGY agar were between 66.6 and 100% while the specificity values changed between 95.4 and 100% for other *Candida* species.
Conclusion: It can be concluded that the use of CHROMagar *Candida* is an easy and reliable method for the presumptive identification of most commonly isolated *Candida* species especially *C. albicans*, *C. tropicalis*, *C. krusei* and *C. glabrata*. On the other hand, BIGGY agar can also be used for the identification of *Candida* species however; its sensitivity and specificity values are lower for the most isolated species such as *C. albicans* and *C. tropicalis*.

P1077 Improving medical outcomes, service outcomes and cost outcomes: implementation of Rapid Respiratory Viral Direct Fluorescent Testing (the Raving Fans Generating Test Process)

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Objectives: 'Between the health care we have and the care we could have lies not just a gap, but a chasm.' Intermountain Health Care (IHC) is an internationally recognized leader in closing this quality chasm. For more than 15 years, we have studied care processes and the consistent delivery of healthcare according to best practice standards. Our objective in this evaluation is to determine if the availability of a sensitive, rapid diagnostic test for respiratory viruses would affect length of stay (LOS)and antibiotic use in a children's hospital.
Methods: A study was performed at Primary Children's Medical Center (PCMC) to determine the adequacy of direct fluorescent assay (DFA) in rapid detection of viral respiratory pathogens. Acquired fundamental knowledge was applied to improve the process using outcomes research techniques. Each nasal wash sample was prepared by cytospin and tested by the following methods: (1) SimulFluor Respiratory Screen (Chemicon International, Temecula CA), followed by SimuFluor FluA/FluB and SimuFluor Para 1,2,3/Adeno stains if warranted, (2) viral respiratory culture using four shell vials with 72 h and 10 day exit stains.
Results: A true positive was defined as any nasal wash specimen that was positive by DFA and by viral culture. Of the 6225 adequate samples, 2177 were true positive for an overall positivity rate of 34.9%. Viral distribution was Respiratory Syncytial Virus ($N = 1433$), para influenza virus ($N = 269$), adenovirus ($N = 146$) influenza A ($N = 275$) and influenza B ($N = 54$). Overall sensitivity and specificity was 86.7 and 93.8%, respectively. Average turn-around-time was approximately 9 h.
Conclusions: With each positive DFA diagnosis, medical outcomes improved by shortening LOS by 1.5 days and administration of less IV antibiotics by 18% ($P = 0.008$) and administration of less oral antibiotics at discharge by 15% ($P = 0.02$). Service outcomes improved by providing a diagnosis within 9 h (vs. 4–5 days) and cost outcomes improved by providing a savings of US $4,000.00 per positive DFA diagnosis.

P1078 Evaluation of a new chemiluminescent assay for the detection of Epstein-Barr virus antibodies

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Objectives: Epstein-Barr virus (EBV) infects and persists for life in the majority of the human population which is developed in several levels, from the acute infectious mononucleosis to reactuvation of the latent virus. We have evaluated a new chemiluminiscent assay for to determine the profile anti-EBV antibodies and the EBV infection status.
Methods: Two hundred and forty-eight sera were selected from several situations defined by clinical and analytical criteria: not infection (27), acute infection (56), past infection (79) and reactivation (86). Anti-EBV antibodies (VCA IgG, EBV IgM, EBNA IgG, EA IgG) were quantitatively determined by a automation chemiluminiscent assay (Liaison, Diasorin).
Results: From 27 EBV(–) patients, 21 were negative markers and 6 were EBNA IgG close to cutoff. In samples corresponding to acute mononucleosis, 51 were EBV IgM (91.0% sensitivity and 90.9% specificity) with negative detection of anti-EBV IgG (VCA, EBNA or EA). For the detection of present or past EBV infection, sensitivity, specificity, and positive and negative predictive value were 100, 77.7, 97.3 and 100, respectively. EBV reactivation was a close relationship with the quantitative value of anti-EBNA because over 600 IU/mL the positive predictive value was 88.6, falling to 69.3 and 61.8 when over 200 and 100 IU/mL, respectively, were detected. From 55 sera with past infection studied, only 2 positive and 6 in gray zone EA IgG were detected, corresponding tocases of subacute infection, with EBV IgM negative and ENBA IgG low or negative value.
Conclusions: The detection of EBV antibodies by chemoluminiscent Liaison assay is a new automated method very useful for to know EBV infection status. Their advantages are speed, automation, simplicity, quantitative measure, run high workload and good sensitivity to detect acute infection. As disadvantages we found very difficult to detect EBV reactivation however, is possible to demonstrate past reactivation when EBNA IgG titer s very high. The implementation of avidity test for to VCA IgG can be to improve the discrimination between subacute or past infection, when EA IgG shows unfinished result.

P1079 *Chlamydia pneumoniae* pediatric serology: comparison between two assays

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Objectives: Serological analysis represents the current routine method for the diagnosis of *C. pneumoniae* infections because the direct research by PCR is difficult. The microimmunofluorescence assay (MIF), which requires expertise to perform, was considered, for a long time, the 'gold standard method'. Now, automated enzyme-linked immunosorbent assays (ELISAs), which are more standardized and for which the reading of results is less subjective, have been developed for routine diagnosis. The aim of our study was a comparison between two assay methods (ELISA and MIF), for the determination of anti-*C. pneumoniae* antibodies in paediatrics.
Methods: We selected serum samples from children (2–14 years) during normal laboratory routine: 104 children with high or low respiratory tract infections that showed high serum level *C. pneumoniae*-specific IgM and/or IgG antibodies in MIF (SeroFia, Savyon, IL), and 114 sera from apparently healthy children without antibodies (according to MIF) against *C. pneumoniae*, *C. trachomatis*, *C. psittaci* which were used as a control group. *C. pneumoniae*-specific IgM and IgG antibodies were also determined in these two groups of serum samples by a commercial ELISA test (Cp-Eurospital, I). The tests were performed and analyzed as recommended by the manufacturer.

Results: Results show the specific concordance of the presence of anti-*C. pneumoniae* IgM and IgG antibodies in different patient groups determined by two assays. There was a very high concordance (98%) between the results of the two different methods.
Conclusions: The results obtained by ELISA overlap those obtained by a traditional MIF assay and show that the ELISA test is highly reliable. In addition, the rapidity, the ease of use, the possible automation as well as the easy analytical interpretation independent of the subjective evaluation of the operator, are important advantages.

P1080 Tau- and S100B-protein but not phospho-tau in the cerebrospinal fluid are higher in bacterial than in viral infections of the central nervous system

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Objectives: In patients presenting with fever and meningism, the differentiation of bacterial from viral central nervous system infections often remains clinically uncertain, despite of additional and widely accessible laboratory parameters. S100B, tau and phospho-tau (ptau) all are proteins being released in various kinds of brain damage. The concentrations of the glial protein S100B in the serum and cerebrospinal fluid (CSF) predict the outcome after hypoxic brain damage. The neuronal proteins tau and ptau help in the differential diagnosis of dementia. This pilot study aims to evaluate the pattern of S100B, tau and ptau release in infections of the nervous systems and their potential value in the differential diagnosis algorithm of viral and bacterial infections.
Methods: CSF of patients with clinically suspected CNS-infection was collected consecutively over 3 months whenever material was left from routine diagnostics. After the diagnostic workup had been finished, we excluded CSF of fungal-, borrelia- and HIV-infected patients. A total of 29 specimens remained, $n = 12$ viral and $n = 17$ bacterial infections, respectively, for analysis. S100B, tau- and ptau were measured using commercially available ELISA-kits.
Results: Both S100B and tau-protein were higher in the CSF of patients with bacterial than in those with viral infections ($P < 0.05$). In contrast, the release of ptau in both groups did not differ.
Conclusion: This preliminary study shows that S100B- and tau-protein, but not ptau protein could be helpful candidates for the differentiation of bacterial from viral central nervous system infections.

P1081 Comparison of TestPack RSV and Directigen RSV with direct immunofluorescence assay

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Objectives: In the present study, we compared two rapid diagnostic techniques, Testpack RSV and Directigen RSV with direct immunofluorescence assay (DFA), for Respiratory Syncytial Virus (RSV) detection.
Methods: Fifty nasopharyngeal aspirates collected from children aged <2 years, with acute lower respiratory tract infection, were evaluated by Testpack RSV and Directigen RSV, for RSV detection. Thirty-six specimens were found to be RSV positives and 14 RSV negatives, when tested with DFA. The number of fluorescing cells per optical field (fc/pof) was counted for the RSV positive specimens, in order to discriminate low (0–2), medium (2–4) and high (> 5) positives. After DFA testing, all specimens were kept frozen at − 8 °C for up to 3 months before evaluation.
Results: No false positive results were accounted by Test pack RSV and Directigen RSV. The specificity and positive predictive value for both Tests vs. DFA for RSV detection, was 100%, but their sensitivity ranged from 56 to 80% and 100% when low, medium or high RSV positivity specimens were tested.
Conclusions: Negative results by Test pack RSV or Directigen RSV should be retested with a more sensitive method, such as, PCR, cell culture, or IFA, which are more expensive, time consuming or require experienced personel.

P1082 A rapid modified antibody-capture enzyme-linked immunosorbent assay for detection of HSV-1 primary infection in humans

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Objective: Development and application of an enzyme-linked immunosorbent assay (ELISA) for detection and measurement of IgM antibody to herpes simplex virus type 1 (HSV-1) in human sera.
Methods: An antibody-capture ELISA based on the reaction between antihuman IgM, prepared in rabbits, and IgM of serum samples and HSV-1 conjugated with horesradish peroxidase was developed and applied. Five hundred sera from healthy persons and 26 sera from persons with primary HSV-1 infection were tested for the presence of IgM antibody against HSV-1. The specificity and sensitivity of the test were compared with a commercial test kit, and was acceptable.
Results: Based on the results obtained from this method, it was shown that this method is suitable to detect IgM to HSV-1. Seven point three percent of healthy sera were positive for IgM. The sensitivity and specificity of the test was measured and were 94.4 and 93.91%, respectively.
Conclusion: Detection of HSV-1 IgM provides a sensitive, rapid and economic method for the diagnosis of HSV-1 primary infection. The ELISA test developed in this study had acceptable sensitivity and is a suitable method for the detection of HSV-1 primary infection.

P1083 IgG avidity for toxoplasmosis detection by the Liaison system

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Objectives: To evaluate the usefulness of IgG avidity detection with an automatic system for toxoplasmosis diagnosis and to diferentiate between acute and past infection.
Methods: Eighty-nine serum samples collected between 1999 and 2002 from 20 patients diagnosed with probable toxoplasmosis, in whom IgM detection was positive in a number of the sera were selected retrospectively. ELISA IgG detection and capture ELISA IgM detection were determined by the access system. Positive results for IgM were confirmed by the Vidas system (inmunocapture). IgG avidity was determined by the Liaison system.
Results: Nine out of the 20 patients with IgM positive (40 sera) had high IgG avidity in all sera, indicating a past infection. Three patients (10 sera) showed an IgG and IgM seroconversion and their IgG avidity changed from low to high. In three patients (17 sera) IgG avidity remained low during few months (between 5 and 10 months).
Conclusions: IgG avidity test has demonstrated its usefulness to rule out an acute toxoplasmosis. Long standing of low avidity IgG in sera of some patients diminish its value to differentiate between acute and past toxoplasmosis, being necessary complementary detections.

P1084 Performance and clinical use of stage-specific toxoplasma antigens investigated with the new DPC Western blot assay

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We evaluated the performance of the new DPC Western blot for toxoplasmosis on 123 sera from 103 adults and on 12 samples from 7 newborns presumed to be congenitally infected. Clinical aspects and immune status in the adults were supported by a panel of seven methods focusing on the combination of isotypes IgG, IgM and IgA with avidity. The relative specificity, in terms of absence of infection, was 100% (30/30) in individuals with negative IgG and negative IgM antibodies, 100% (4/4) for reactivation and 96% (28/29) for past immunity. The relative sensitivity was 100% (46/46)

in the infected group. The seropositivity criteria for the binding of IgG were P30 and P35 for both early seroconversion ($n = 2$) and recent infection <3 months ($n = 36$); P30, P35, P20, P24, P63 with P55 for acute infection >3 months ($n = 8$) and without P55 for past immunity ($n = 29$). P30 IgM was the major criterion within the infected group, whereas the group with past immunity did not show any IgM reactivity. Following seroconversion during pregnancy, the comparative mother–infant immunological profiles revealed newly synthesized IgG antibody at birth in one child and at 3 months in another child. In contrast, IgM and IgA immunocapture remained negative in both cases. We thus recommend the DPC Western blot as a first line-assay for early diagnosis of postnatal congenital toxoplasmosis and as a second-line confirmatory assay for past immunity vs. infection in adults, in particular, when an IgM antibody has been detected by the conventional techniques.

P1085 A rapid, simple and inexpensive Fluorescent In Situ Hybridization (FISH) assay for direct detection of *Babesia microti* on a blood smear

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Babesiosis is a tick-borne disease caused by an intraerythrocytic parasite. At present, four species of Babesia, *Babesia microti*, *Babesia divergens*, *Babesia MOI* and *Babesia*-WA1 are known to infect man. *B. microti*, *B. MOI*, and *Babesia-WA1* are present in the US. *B. divergens* and *B. microti* are present in Europe. The Giemsa-staining of a whole blood smear is the method of choice for diagnosing Babesiosis. However, it is neither sensitive nor specific. The introduction of PCR to the field has resulted in greater specificity and sensitivity, but the time and cost requirements are still not optimal. Therefore, we have developed a highly specific, inexpensive and simple fluorescent in situ hybridization (FISH) test for detection of *B. microti* directly on an air-dried blood smear. The assay can be performed in less than 1 h. An added advantage is that the assay provides parasite morphological information. The ribosomal RNA of *B. microti*, within the fixed blood smear (on a glass slide), is hybridized to a fluorescent-labeled oligomer probe that is highly specific for *B. microti*. The excess probe is washed off. The processed smear is counterstained and viewed at 40× under UV microscope using fluorescent specific filter set. The *B. microti* parasites if present fluoresce apple-green within the red blood cells. Over the past 2 years we received 25 blinded blood samples from patients with Babesiosis-like symptoms from the New York State Department (NYS). Smears prepared from EDTA whole blood of 21of the 25 patients, were tested by the FISH assay. NYS confirmed all 21 samples by Giemsa staining and PCR. Seven were *B. microti* positive and 14 were *B. microti* negative. Of the 14 *B. microti* negative samples, 3 were from *Plasmodium falciparum* positive patients and 2 were from *Trypanosoma cruzi* positive patients. All 14 *B. microti* negative samples were negative by the FISH assay. Of the 7 positives, 6 were correctly identified by the FISH assay. The one sample that was missed by the FISH assay was from a patient whose blood was at 4 °C for more than 5 months before the smear was made. Thus, the FISH assay had a specificity of 100% and sensitivity of 86%.

Molecular diagnosis and characterization of Gram-negative bacteria

P1086 A combined AFLP – multiplex PCR assay for molecular typing of *Escherichia coli* strains using variable bacterial interspersed mosaic elements (BIMEs)

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Objective: The original method for molecular typing of *E. coli* strains was developed using the polymorphisms in chromosomal sequences of bacterial interspersed mosaic elements (BIMEs) detected by multiplex polymerase chain reaction (PCR) and analyzed by amplification fragment length polymorphism (AFLP) assay.
Methods: The method was based on detection of the patterns obtained by the combinations of three different amplification products of variable sequences localized between the genes for mtlA and mtlD, lamb and malM, araA and araD. The three primer pairs were targeted to the conserved sequences of 3′ and 5′ ends of these genes to amplify variable intergenic sequences. The lengths of these amplicons were variable due to strain-specific arrangements of noncoding intergenic sequences BIMEs and boxCs, which are located between relatively conserved genes. The applicability of the method as an epidemiological tool was tested on the group of 350 *E. coli* strains cultured from beef meat, milk and milk products and on 174 *E. coli* strains cultured from human feces and urine.
Results: The observed fragment lengths varied from 170 to 554 bp and were combined producing 18 genotypes (A–S) from which some were preferentially detected in certain materials (genotype J in human urine, genotype C in human feces and in cow milk). Significant differences were found in the frequencies of these genotypes among various groups of different origins (milk farms 1 and 2).
Conclusion: From the data here presented we could conclude that this method can reliably detect and recognize various *E. coli* genotypes. The presented method when tested on bigger groups of epidemiologically different sources could be promising tool for simple, rapid, robust and reproducible molecular epidemiology of *E. coli*.

P1087 Use of real-time PCR for rapid detection of *Salmonella* spp.

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Objectives: The development of a rapid and reliable real time PCR assay, that uses a single-tube amplification, for the detection and quantification of *Salmonella* organisms and its application for the rapid detection of *Salmonella* spp. in mayonnaise samples.
Methods: A pair of primers were used to amplify a 457-bp fragment that overlaps the junction between the invE and invA genes, and for detection we used either Sybrgreen I or two specific labeled probes that hybridize with adjacent sequences in the amplicon when present in the reaction mixture. Ninety-two strains of 35 different *Salmonella* serovars and 77 strains belonging to 34 non-*Salmonella* species were studied.
Results: The sensitivities of both assays were higher than those of previously described PCR protocols; 5 fg of DNA/PCR for the Sybrgreen method and 10 fg of DNA/PCR were detected for the hybridization method. The specificities were 100% for both systems. We were also able to detect 1 cell in 25 g of food product after 7 h of preenrichment in buffered peptone water (BPW), with both LC Sybrgreen and LC FRET PCR.
Conclusions: The capillaries tubes do not have to be reopened for post-PCR analysis, the risk of carryover contamination is minimized. With the Light-Cycler methods, all the strains were correctly identified within 30 min without the need of any post-PCR sample manipulation. Overall, this pilot study has revealed that the LightCycler technology is an extremely rapid and economical method for the detection of *Salmonella* organisms.

P1088 Rapid and highly sensitive detection of *Pseudomonas aeruginosa* by real-time, on-line PCR using the LightCycler instrument

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Objectives: *Pseudomonas aeruginosa* is an opportunistic pathogen that causes a variety of localized infections, e.g. of the urinary tract, respiratory system, soft tissue, endocarditis, and a variety of systemic infections, particularly in burn patients and immunosuppressed patients. *P. aeruginosa* has emerged as one of

the most important nosocomial pathogens causing more than 10% of all nosocomial infections, as this organisms is inherently resistant to common antibiotics and even survives in antiseptics.
Methods: We have developed a real-time, on-line PCR-based assay to specifically detect *P. aeruginosa* in nucleic acid preparations from bacterial cultures, blood cultures and biological samples for research use.
Results: Using the LightCycler Instrument for amplification and simultaneous detection of the target DNA we were able to detect *P. aeruginosa* over a seven log 10 dynamic range with an analytical sensitivity of less than 10 genome equivalents per reaction (Probit analysis; 95% cut-off value). Specificity of the assay for *P. aeruginosa* was demonstrated by analysis of 90 *P. aeruginosa* isolates and 100 other Pseudomonads or unrelated organisms. Inclusion of an internal control that is amplified/detected simultaneously in the same PCR reaction prevents misinterpretation of false negative results caused by PCR inhibitors or due to inefficient nucleic acid extraction. In addition, the closed-tube homogenous detection format eliminates the necessity of post-PCR analysis and the risk of carry-over contamination.
Conclusions: The assay provides a new research tool for rapid, highly specific and sensitive detection of *P. aeruginosa* DNA. The assay is especially adapted to the PCR Workflow System (MagNA Pure LC and LightCycler Instrument) thus enabling automation of the complete isolation/detection process from the sample to the result under standardized conditions.

P1089 Comparison of phenotypic and molecular methods to identify multiresistant bacteria from cystic fibrosis patients

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Objectives: It is essential to accurately identify Gram-negative non-fermenting bacilli in cystic fibrosis (CF) patients as both their clinical significance and interpretation of susceptibility test results differs. The aim of this study was to compare routinely performed phenotypic identification with molecular identification methods.
Methods: 90 Gram-negative non-fermenting bacilli were identified by API and other phenotypic methods as well as by species specific PCR. Previously described primers were used to identify the 23S rRNA gene in *Stenotrophomonas maltophilia*, the 16S rRNA gene in *Alcaligenes xylosoxidans* and *Pseudomonas aeruginosa* and the recA gene in the *Burkholderia cepacia* complex.
Results: The majority of the isolates (59) originally identified phenotypically as *P. aeruginosa*. Of these 91.5% confirmed by PCR. The remainder were identified using PCR as either *A. xylosoxidans* (3 isolates) or *S. maltophilia* (2 isolates). Of the 19 isolates which were phenotypically identified as *B. cepacia*, 10 were confirmed by PCR. The remainder were identified by PCR as *S. maltophilia* (6 isolates), *A. xylosoxidans* (1) or *P. aeruginosa* (1) with one isolate remaining unidentified. All three of the isolates identified phenotypically as *A. xylosoxidans* were confirmed by PCR. All nine of the isolates identified phenotypically as *S. maltophilia* were confirmed by PCR.
Conclusions: 84.4% of the isolates were identified as the same species by both phenotypic and PCR-based methods. Of the 14 isolates incorrectly identified by phenotypic means, 57% were identified as *S. maltophilia* and 29% identified as *A. xylosoxidans* by PCR.

P1090 Genetic diversity of *Burkholderia cepacia* strains isolated from cystic fibrosis patients in Torino (North-west Italy)

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Objectives: Forty-one *Burkholderia cepacia* strains, among which 33 isolated from cystic fibrosis patients (children and adults) in Torino (Italy) and 8 reference strains (obtained from different sources) were assessed to evaluate their: (i) genetic characteristics (using PCR, PFGE and RAPD techniques); (ii) transmissibility (by detection of the cblA and BCESM genes) and (iii) antibiotic susceptibility.
Methods: The strains were biochemically identified (Vitek auto microbic system and API 20NE). By PCR with specific primers, their genomovar status and the presence of genes responsible for the expression of epidemic markers were tested. Two genomic typing systems, PFGE and RAPD, were used to further discriminate the genotypes. The antibiotic susceptibility was analyzed using the Vitek AMS System and the disc diffusion method.
Results: Among the isolated *B. cepacia* strains, 23 (70%) were characterized as genomovar III-A and 3 (9%) as genomovar VII. No strains belonging to genomovars I, II, III-B, IV, V and VI were identified, and 7 strains were grouped in other not yet identified genomovars. Only one of the isolates, belonging to the genomovar VII, was positive for the epidemic gene cblA, whereas 15 (5 from children, 10 from adults, all genomovar III-A) were positive for the epidemic marker BCESM. Analyzing the chromosomal DNA with PFGE and RAPD techniques combined, 6 genotypes were differentiated; only two of these were found both in children and adults. The isolates belonging to the genomovar III-A were distinguished in 3 genotypes and two of these presented the BCESM gene. In all isolates we revealed a marked resistance to the aminoglycosides amikacin and tobramicin, to the beta-lactams carbenicillin, piperacillin and ceftazidime. In particular, in 8 of the 12 strains resistant to all these antibiotics the gene BCESM was demonstrated.
Conclusion: The major part of the *B. cepacia* strains isolated from cystic fibrosis patients belonged to the genomovar III-A (70%) and were positive for the BCESM gene (45%). Using PFGE and RAPD, we demonstrated that these were grouped into three peculiar types that differ from strains isolated in other countries. Moreover, there appears to be a link between the presence of the BCESM gene and resistance to antibiotics, suggesting a transmission of genetic virulence factors.

P1091 Combined analysis of AFLP- and REP-PCR patterns to determine episodes of potential transmission with *P. aeruginosa* in five intensive care units

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Background: Standardized PCR-based methods are missing to genotype numerous isolates of nosocomial pathogens. Easy-to-use methods like RAPD techniques do not offer a high enough reproducibility. Sequence-based procedures require a high laboratory expertise and are not fully evaluated for the microepidemiological setting.
Objective: To evaluate a combined analysis of Amplified Fragment Length Polymorphism (AFLP)- and Repetitive Element PCR (REP PCR)-method determining episodes of potential transmission (EPT) with *P. aeruginosa* (P.a.).
Methods: A prospective study was performed in five ICUs of a university hospital over a period of 18 months with surveillance of all nosocomial infections. All clinical P.a. isolates were collected from patients having had more than 48 h stay in the ICU. Community acquired P.a. isolates detected in patients from the same region were also typed. REP-PCR with ERIC2 primers and an AFLP protocol with restriction enzymes *Eco*RI and *Mse*I were used. Automated laser fluorescence analysis of the PCR products was generated in a DNA sequencer (ALF II). Cluster analysis was performed with Gel Compar II using UPGMA and the Pearson coefficient. Isolates were assigned to the same strain if the percent genetic similarity exceeded 80% in both PCR methods. One *n* isolates from different patients of the one clone yielded in *n* minus 1 EPTs. For these patients their ICU stay had to overlap or not exceed 9 days between discharge of the one patient and admission of the other patient.
Results: A total of 1769 different patients with 28 498 patient days were included. 184 P.a. isolates were genotyped. The genetic similarity of all type and reference strains in different gel runs exceeded the threshold. After exclusion of copy strains from different sites of the same patient 134 P.a. isolates remained. 10–39 different P.a. strains were detected in the five ICUs. 84 community acquired isolates (68 strains) were genotyped. The genetic diversity of the P.a. isolates from both sources did not differ significantly. 18 EPT were detected. 34 nosocomial infections due to P.a. were identified and 5 were associated with EPT.
Conclusion: Isolates of P.a. had a high clonal diversity, detection of identical strains in different patients is possibly due to transmission. 14.7% of nosocomial P.a. infections were associated with EPT. A combined analysis of AFLP- and REP-PCR method may be a useful tool to determine transmission with *P. aeruginosa*.

P1092 Molecular typing using arbitrarily primed polymerase chain reaction: establishing an epidemiological relationship between nonfermentative Gram-negative rods involved in an outbreak of pseudobacteremia

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Objectives: A case–control study performed previously in our hospital indicated potential contamination of blood culture (BC) bottles by nonfermentative Gram-negative rods originating from the liquid content of the erythrocyte sedimentation rate (ESR) tubes. The purpose of the study was to investigate the epidemiological relatedness of these strains by arbitrarily primed polymerase chain reaction (AP-PCR).
Methods: Fifty isolates from BC bottles and seven from ESR tubes were collected during the year 2000. In addition 16 epidemiologically unrelated strains were examined. Study isolates were identified by Phoenix automated microbiology system (Becton Dickinson) and API 20 NE (BioMerieux). For AP-PCR the following three primer sets were used: M13 core, DAF4 and ERIC1R/ERIC2R.
Results: Based on AP-PCR five clonal groups (I–V) could be identified, with isolates displaying similar fingerprints within each group. The predominating species in these groups were as follows: I and II *Ochrobactrum anthropi*, III *Stenotrophomonas maltophilia*, IV *Pseudomonas stutzeri* and V *Chryseobacterium meningosepticum*. In three of these five groups identical isolates from ESR tubes and BC bottles were identified. All 16 unrelated strains showed different fingerprints, suggesting a high discriminatory power. Typeability was best for primer sets DAF4 (98.6%) and M13 core (97.3%).
Conclusion: The results are consistent with our hypothesis that ESR tubes were the source of the pseudobacteraemias. AP-PCR with primer sets DAF4 and M13 core can be used as a rapid and simple typing method for nonfermentative Gram-negative rods.

P1094 Detection of *E. coli* O157:H7 stx1 and stx2 genes by oligochromatography of PCR amplicons

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Enterohemorrhagic *Escherichia coli* (EHEC) produces different protein exotoxins that play an important role in the pathogenesis of diarrheal diseases. Infection by Shiga-like toxin (ST)-producing *E. coli* may result in hemorrhagic colitis with the possible development of hemolytic uremic syndrome. STEC strains are difficult to distinguish by cultivation methods, except for *E. coli* 0157:H7. They are more reliably identified through the detection of their toxin genes. Several methods for the simultaneous PCR detection of these genes have been described; analysis of the amplicons were primarily done by gel electrophoresis that differentiates the amplicons on their lengths. This method cannot exclude false-positives with similar lengths as one of those expected. Specific detection requires effort and time-consuming hybridizations with an internal probe either by blotting or hybridization-coupled immunoassays. More recently, real-time amplification techniques enabled to detect specifically different genes, but with the use of expensive equipment. In order to perform easy and specific detection of multiplex PCR-amplified stx genes encoding the *E. coli* STs, we set up and tested a new detection method that we call oligochromatography which is similar to immunochromatography. SLT-I and II gene amplification was performed by PCR with one hapten-labeled primer for each gene. Biotin was used for stx1 and DIG for stx2. For each amplicon, an internal probe was conjugated to colloidal gold particles. Oligochromatography is performed by placing a stick in the PCR product. While migrating, the amplicon reacts with the dried conjugated probe and then accumulates on a line where an antihapten antibody is coated, giving rise to a red line in less than 5 min for high positives. Oligochromatography was compared with agarose gel with ethidium bromide staining for detection of PCR amplified stx genes from EHEC strains. Oligochromatography was shown to be more sensitive and quicker than agarose gel. Results are observed in less than 10 min following the end of amplification. Oligochromatography could be regarded as a good alternative to current methods since it gives rise to rapid and specific detection of amplified products without the need of extra material.

P1095 Assessment of interlaboratory reproducibility of genomic fingerprints of *Acinetobacter baumannii* by the Acinetobacter Working Group of the ENEMTI network funded by ESF

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Objectives: To validate methods for electronic exchange of Acinetobacter genomic fingerprints for establishment of an Internet-based database to study the geographic spread of clinically important strains.
Methods: Three methods were explored, RAPD-fingerprinting, pulsed-field gel electrophoresis (PFGE), and AFLP high resolution genomic fingerprinting. Twenty-seven *Acinetobacter baumannii* strains were used including outbreak and sporadic strains. Results of the participating laboratories were centrally analyzed using the BioNumerics software package (Applied Maths, Sint-Martens-Latem, Belgium).
Results: RAPD profiles of a training set of 6 strains generated with DAF4 as primer, Ready-to-Go RAPD beads and separation in 2% agarose gels were not compatible between laboratories. PFGE fingerprints of the 27 strains were generated in 3 participating laboratories with *Apa*I as restriction enzyme, followed by fragment separation in 1% Seakem Gold agarose for 19 h with pulses ranging from 5 to 20 s. Central analysis with lambda ladder standard for normalization showed 100% matching of strains, but the tolerance setting was relatively high due to the limited set of marker lanes and the low number of bands in the fragment size zone used. Results of multiple samples of one Acinetobacter strain (RUH2034) indicated that use of this strain as standard for normalization may allow a tolerance setting of 1.5%. Comparison of AFLP results between two participating laboratories with different fragment separation systems indicated that interlaboratory data exchange was not possible.
Conclusion: Pulsed field gel electrophoretic fingerprints of *A. baumannii* strains generated according to the used protocol have the potential to be used for electronic data exchange and establishment of an Internet-based database.

PCR diagnosis of difficult to grow organisms

P1096 DNA amplification of *Leptospira* spp. by polymerase chain reaction in the diagnosis of leptospirosis

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Objectives: This study is aimed to compare PCR sensitivity between two pairs of primers (G1/G2, Gravekamp et al., 1993 and LP1/LP2, Sun Ho et al., 1994) in blood and urine samples from patients with leptospirosis; to study PCR specificity in samples in healthy individuals and patients with other diseases; to compare PCR results with the microagglutination (SAM), IgM ELISA and the macroagglutination test (SAT).
Methods: Among the 60 patients screened, 33 had confirmed diagnosis of leptospirosis with seroconversion through microagglutination test and/or positive hemoculture and 27 patients with antibodies titers $\geq$ at 800, according to WHO (Faine, 1994). The control group was constituted by 44 healthy individuals with nonreactive microagglutination test and 20 patients with other diseases, confirmed by microbiologic, parasitologic and/or immunologic tests. The following tests were applied for statistical analyses: χ^2 test with Yates correction, Fisher exact test if necessary and McNemar test for evaluation of response between two dicotomic variables with the significance level of 5% ($P = 0.05$).
Results: Sensitivity with both primers was 10 pg in agarose gel and 1 pg by hybridization. In blood and/or urine samples these primers were able to amplify DNA in 39 out of 60 patients with leptospirosis (65%). Use of primers

G1/G2 (60%) proved to be more sensitive in comparison to LP1/LP2 (30%)($P = 0016$). Urine samples presented greater sensitivity (45.8%) with primers G1/G2 in relation to blood samples (31.7%) in patients with leptospirosis. However, greater sensitivity was observed in blood samples (21.7%) with primers LP1/LP2 than in urine samples (12.5%). The PCR with each of the primers proved to be specific, not amplifying DNA of other bacteria, from healthy individuals, or from patients with other diseases. Comparing serologic tests and PCR on patient's admittance (first sampling) the IgM ELISA test was the most sensitive, followed by macroagglutination and microagglutination test and PCR. The specificity of the tests was in decreasing order microagglutination, PCR, macroagglutination and IgM ELISA.
Conclusion: In conclusion, PCR is an useful and specific technique for the diagnosis of leptospirosis and was the only technique able to confirm this diagnosis in the initial stage of the disease in 4 out of 11 patients presenting negative results for all serologic tests. Supported by: FAPESP and Laboratory of Immunology (LIM 48), Clinic Hospital, School of Medicine, São Paulo University.

P1097 Polymerase chain reaction assay specific for pathogenic *Leptospira* based on the gene encoding the hemolysis-associated protein (Hap1): identification of the primers Adia 217 and 218®

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Objectives: Leptospirosis is a spirochetal disease with multiorgan involvement. The clinical presentation is often a diagnostic challenge because of its manifestation and varying severity. As pathogenic leptospires are very tedious in culture, PCR is a more useful tool for the diagnosis of leptospirosis. Lep1-Lep2 primers (Merien) are often used in clinical samples and are genus specific. As animal samples would be often contaminated, it would be interesting to differentiate the pathogenic from the saprophytic species belonging to *Leptospira* genus. Two primers named Adia217 and Adia218 were selected from the gene hap1 (AF366366) of the Hemolysin-Associated Protein (designated as LipL32 by Haake). The presence of hap1 gene were analyzed by DNA/DNA hybridation (SB) and PCR was performed either on *Leptospira* spp. cultures either on clinical and water samples.
Methods: DNA was extracted by Qiagen process from 17 cultures of pathogenic, saprophytic and intermediate strains of leptospires. Blood, urine samples and kidneys were collected from dogs with leptospirosis and submitted to culture and PCR with Adia217-Adia218 after DNA extraction. PCR analysis of concentrated water samples was performed by both primers lep1-lep2 and Adia217-Adia218 too.
Results: Cultures of *L. interrogans* ss serovars *pomona* and *icterohemorrhagiae*, *L. kirschneri* serovar *grippotyphosa*, *L. borgpetersenii* serovar *javanica*, *L. noguchii* serovar *panama*, *L. inadai* serovar *lyme* gave a positive signal in PCR confirmed by SB whereas cultures of *L. fainei* serovar *hurstbridge*, *L. meyeri* serovars *semaranga* and *ranarum*, *L. wolbachii* serovar *codice*, *L. biflexa* ss *serovar patoc*, genomospecies 5 serovar *sao paulo* and *L. illini* gave a negative signal in PCR confirmed by SB. The results are discussed in term of sensitivity of culture compared with the threshold of PCR.
Conclusions: PCR with Adia17-Adia18 primers can be useful to differentiate pathogenic from saprophytic leptospires. This rapid specific test has a potential clinical and prophylaxis applications especially with surveillance of recreational water.

P1098 PCR technique for detection of *Treponema pallidum* ssp. *pallidum*

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Objectives: *Treponema pallidum* ssp. *pallidum* is major human pathogen. Emerging increase of syphilis incidence in some european countries, especially of adnate form, requires improvement of routine diagnostic techniques. As detailed map of *T. pallidum* already exists, nucleic acid detection-based methods are very promising. So far, only a few results were achieved on this field. Our aim was to develop sensitive, specific and user friendly PCR-based laboratory technique for routine diagnostics of syphilis.
Methods: PCR format using DIG-labelled nucleotides and anti-DIG ELISA detection system was chosen because of standardised commercial reagents. Sets of primers and biotin-labelled capture-probes for three genes (polA(I), 47-kDa and bmp) were tested. For test calibration and as positive controls *T. pallidum* strain Nichols—freshly cultured and freeze-dried was used. As negative control served human DNA extracted from lymphocytes. Different systems for sample collection and DNA isolation have been proven for lesion, whole blood, serum/plasma and CSF specimens.
Results: Amplicor STD Specimen Collection Kit (Roche) provided optimal conditions for DNA stability and integrity of Tp from lesions. Isolation results from whole blood samples were equal, using Wizard DNA Isolation Kit (Promega) or Amplicor Whole Blood Preparation Kit (Roche). Amplicor kit seemed to be more user-friendly. Amplification profile and Mg^{2+} concentration have been optimized for PCR reaction. Systems for 47-kDa and polA(I) genes were more effective than bmp one. Fresh Nichols strain functioned best as positive control. Co-amplification-based internal control using system for tpA or beta-actine gene sequences was added to avoid false negative results due to polymerase inhibition. Test sensitivity limit for whole blood was 50 *T. pallidum* cells/mL.
Conclusions: PCR technique was optimised for routine detection of *Treponema pallidum* DNA in lesion and whole blood samples. Standardised sample collection, preparation, amplification and detection reagents and coamplification-based internal control were implemented. Test is rapid, sensitive and user-friendly.

P1099 PCR in Lyme neuroborreliosis—post-treatment progress

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Objectives: High diagnostic value of PCR in neuroborreliosis (NB) is caused by the direct way of spirochete detection. Elimination of DNA after acute NB is poor documented. Prospective study on this topic is being held and the latest results are presented.
Methods: Three sets of primers in nested PCR were used: one for plasmide gene encoding OspC protein and two for chromosomal gene – 16S rDNA and flagellin. So far 57 patients with clinically manifested involvement in NB were enrolled into the prospective study. Patients were included according to main criterions: positive CSF/serum antibody index (in 48 patients) and PCR positivity in CSF (in nine patients). Patients were examined by neurologist and samples of CSF, plasma and urine were taken before treatment and after treatment was urine examined in periods as followed: (1) after treatment (3 weeks of benzylpenicilin) (2) after 3 months (3) after 6 months. The most frequent clinical diagnosis was Bannwarth's syndrome (31), acute encephalitis (14) and poly(radiculo)neuritis (12).
Results: Before treatment were 20 patients PCR positive in CSF, 16 in plasma, and 28 in urine. Five were parallel positive in all three body fluids. Urine after treatment was positive in 17 cases (30%), after 3 months was positive in 8 patients (15%) and after 6 months in 1 (2%).
Conclusions: In the most of patients DNA has been eliminated up to 3 months from urine. The frequency of PCR positivity in urine and CSF is very similar in the period before treatment. The PCR has got relative high sensitivity (46%), but does not reach the sensitivity of antibody index. Supported by grant MZCR 6244; 111300003.

P1100 PCR in whole blood in diagnosis of syphilis

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Objectives: The diagnosis of false-positive or false-negative results of serologic tests in syphilis continues to pose a difficult clinical challenge. Because the serodiagnosis of syphilis has significant limitation, the direct detection of *Treponema pallidum* in suspect blood may serve as an alternate diagnostic strategy. The aims of the present study were to develop polymerase chain reaction (PCR) with use of whole blood for the diagnosis of syphilis and to estimate its sensitivity and specificity.
Methods: The study persons (186 patients, f/m—82/104, mean age 29 ± 7) included: the patients receiving treatment for syphilis in venereologic clinic; the patients independently addressed to venereologic clinic for examination related to syphilis; the persons directed from other therapeutic and surgical hospitals, at which routine serologic examination revealed doubtful results. Control group consisted of pregnant women (16), patients with chronic infectious and skin diseases (27), drug abuse (14) and 42 healthy donors (f/m—19/23, mean age 23 ± 5). A polymerase chain reaction was carried out

with nested primer pairs based on the DNA sequence of the 47–1 and 47–2 kDa gene of *T. pallidum*. PCR was utilized with 1 milliliter of whole blood. To prevent inhibiting influence of hemoglobin a pretreatment of blood samples by the lysing buffer was performed. A complex of serologic tests: FTA-abs and *T. pallidum* immobilization test (TIT) was used as the 'gold standard'. As a results the sensitivity of PCR was 62.5% and specificity was 93.4%. PCR at 21 patients was carried out simultaneously both with serum and with a whole blood. The comparison of received results has shown that the sensitivity of a method at use of serum is reduced practically twice. It allows assuming an opportunity of a sedimentation *T. pallidum* with a deposit at a centrifugation of a whole blood and validity of its use for reaction. In conclusion results of PCR in whole blood application in syphilis detection revealed its high sensitivity and specificity; possibility to obtain rapid results in unclear cases. Other advantage of PCR is opportunity to discriminate between false-positive and false-negative results of serologic tests in immunologically compromised patients. The purpose of the following researches to evaluate the opportunities of PCR application in differential diagnosis of asymptomatic, latent syphilis and false-positive results of serologic tests.

P1101 Rapid diagnosis of *Bartonella* infections using automated DNA extraction and real-time PCR

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Objectives: Several *Bartonella* species are important human pathogens that cause a variety of clinical syndromes. Diagnosis of infections by *Bartonella* species is seriously hampered by their fastidious growth characteristics. We have developed a real-time PCR assay for rapid diagnosis of clinically relevant *Bartonella* species.
Methods: The MagNaPure LC for automated DNA isolation in combination with LightCycler PCR and DNA sequence analysis was used. DNA was extracted from *B. henselae*, *B. quintana* and *B. elizabethae* cultures. Before using the MagNa Pure Bacterial DNA isolation kit III were cells lysed with Proteinase K. PCR primers targeting the 16S-23S rRNA intergenic region of the genus *Bartonella* were used for PCR amplification. Amplicons were detected with meltingcurve analysis and biprobe hybridisation. A *Bartonella* generic biprobe, labeled with the fluorophore Cy5 was used. When this probe binds to complementary sequences the Cy5 is excited by the energy transfer from Sybr Green I. After a positive probe result the amplicon is sequenced for determination of the *Bartonella* species.
Results: With serial dilutions of bacteria the sensitivity of real-time detection using Sybr Green I, was determined at 1.7 CFU of *B. henselae*, 0.7 CFU of *B. quintana* and 0.2 CFU of *B. elizabethae*. Meltingcurve analysis enabled differentiation of the three species. The probe assay was developed as the primers were found to amplify human DNA from clinical samples. The sensitivity of the probes was identical to the Sybr Green I detection. The probe assay detected *B. henselae* in a lymph node from a patient with cat scratch disease. The MagnaPure isolation in combination with the probe assay is now being evaluated in a large number of clinical specimens.
Conclusions: Automated DNA isolation and biprobe LightCycler PCR allowed rapid and sensitive detection of *Bartonella* species and, in combination with DNA sequencing is a promising and accurate method for identification of *Bartonella* infections.

P1102 *Tropheryma whipplei* and gastroenteritis: frequency of positive results in stool samples by polymerase chain reaction

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Objectives: The leading symptoms of the Whipple's disease are weight loss, diarrhea and arthropathy. In the past years a rod shaped bacterium has been associated with this disease. The causative organism was named *T. whipplei* and can be detected in stool samples by PCR. The aim of this study was to investigate the frequency of *T. whipplei*-PCR positive results in stool samples, which were sent to the laboratory physician's private office for routine diagnostics with the alleged clinical symptoms of diarrhea.
Methods: From May 2002 until November 2002, 1086 stool samples with the suspicion of enteritis pathogens were also analyzed for *T. whipplei* by an in house PCR (in cooperation with IMD, Zürich, Switzerland). The samples were sent from South-west-Germany: Ostalbkreis, Stuttgart and adjacent Bavarian Franken.
Results: *T. whipplei*-PCR was positive for 7.2% of the stool samples, whereas only 4.3% *Salmonellae* were found. Other pathogens such as *Campylobacter*, VTEC (verocytotoxin-producing *E. coli*), EPEC (enteropathogenic *E. coli*), ETEC (enterotoxigenic *E. coli*), EIEC (enteroinvasive *E. coli*) and Norwalk (–like) virus were found to a lesser percentage.
Conclusion: *T. whipplei* is a frequent agent of diarrhea. Physicians should always consider it in their differential diagnosis since its clinical presentation may be so variable and since it may be lethal for the patient.

P1103 Molecular diagnosis of Actinomycotic infections

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Objectives: Actinomycosis is a chronic disease characterised by abscess formation, tissue fibrosis and draining sinuses. It is caused by nonspore forming species of the genus *Actinomyces*. Identification of *Actinomyces* spp. is notoriously difficult, time consuming and unreliable, yet clinically important. Identification in clinical laboratories is based solely upon a limited range of conventional biochemical tests. The aim of this study was to develop more reliable diagnostic methods for identification of *Actinomyces* spp.
Methods: A total of 49 clinical *Actinomyces* isolates, taken from different anatomic sites of infection were examined. A PCR-RFLP method was developed for the identification of *Actinomyces* clinical isolates.
Results: Analysis of the *Actinomyces* clinical isolates with routine laboratory methods identified 45 *A. israelii*, 2 unidentified *Actinomyces* species, 1 *A. meyerii* and 1 *Bifidobacerium* spp. Analysis of the RFLP patterns resulted in the classification of 17 *A. israellii*, 16 *A. gerencseriae*, 13 *A. voscosus*, 2 *A. naeslundii* and 1 *A. bovis* isolate.
Conclusions: The PCR-RFLP method identified a greater species diversity within the panel of isolates tested when compared with current laboratory tests. Many infections caused by *Actinomyces* species are probably misidentified, the use of PCR-RFLP technology should improve the diagnosis and treatment of Actinomycotic infections and our understanding of this chronic disease.

Anaerobic bacteria

P1105 Lactobacilli prevent antibiotic-associated disturbances in the intestinal tract

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Objectives: To compare the effects of clindamycin on the intestinal microflora in subjects ingesting yoghurt with added probiotic microorganisms with the microflora in subjects ingesting placebo yoghurt.
Methods: Twenty-four healthy subjects were included in the study. None of them had taken any antimicrobial drugs during the three months preceding the study. All subjects received 150 mg clindamycin q.i.d. for 7 days and 250 mL yoghurt b.i.d. for 14 days. The subjects were randomized into two groups in a double-blind fashion. Twelve subjects received yoghurt containing 108 cfu/mL of each of the strains *L. acidophilus* NCFB 1748, *B. lactis* Bb12 and Lactobacillus F19 while individuals in the placebo group received yoghurt with no additions. Stool specimens were collected before the start of administration (days −3 and 0), during the clindamycin administration (days 2, 5 and 7) and after the administration (days 10, 14 and 21). The specimens were diluted 10-fold in reduced media and inoculated on selective and nonselective media. The plates were incubated aerobically and anerobically. All morphologically different isolates were identified to genus level. The clindamycin inhibitory concentrations (MIC) for strains of *Bacteroides* spp. collected on days 0, 7 and 21 were determined by the agar dilution method.
Results: In the aerobic intestinal microflora the numbers of enterococci increased after treatment in both groups while other Gram-positive micro-

organisms decreased. In both groups the numbers of *E. coli* also decreased while there was a concomitant increase of other Gram-negative rods, mainly *Klebsiella* spp. In the anerobic microflora in the active group the numbers of lactobacilli and bacteroides remained stable while the numbers decreased in the placebo group. Numbers of bifidobacteria decreased in both groups. Smaller decreases were observed in the numbers of eubacteria, clostridia and veillonella. In both groups there was an increase in MIC values for bacteroides on day 7 that remained on day 21.
Conclusions: The probiotic microorganisms tested in this study prevented ecologic disturbances in the dominating intestinal microflora during clindamycin administration.

P1106 The susceptibility patterns of bacteroides species isolated in two different hospitals

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Objectives: Bacteroides species are the most clinically important anerobic pathogens due to the facts that they are the most frequently isolated anerobic bacteria and they have the widest range of antibiotic resistance. The objective of this study was to analyze the susceptibility profiles of Bacteroides species isolated in two hospitals located in different countries and compare the results. At the same time the results may help for the decision of empirical therapy.
Methods: A total of 36 Bacteroides strains (6 *B. uniformis*, 3 *B. fragilis*, 2 *B. ovatus*, 2 *B. ureolyticus*, 1 *B. vulgatus*, 1 *B. caccae*, 1 *B. eggerthii* from Izmir, Turkey; 20 *B. fragilis* from Edinburgh, Scotland) isolated from clinical specimens in two hospitals were included in this study. The susceptibilities of these bacteria against imipenem, clindamycin, amoxycillin clavulanate, cefoxitin, metronidazole and ciprofloxacin were determined by *E*-test using Brucella agar supplemented with horse blood, vitamin K and hemin. NCCLS breakpoints were used for susceptibility categorization.
Results: The MIC ranges obtained for *B. fragilis* isolates from Edinburgh were <0.002–0.5, 0.016 to >256, <0.016–32, 0.023–64, 0.023–0.38 and 1.5 to >32 μg/mL for imipenem, clindamycin, amoxycillin clavulanate, cefoxitin, metronidazole and ciprofloxacin, respectively. These values were 0.064–1.5, 0.064–0.38, 0.19–2, 2–32, 0.064–0.19, 0.25–24 μg/mL for *B. uniformis* strains and 0.064–0.125, 0.094–0.25, 0.19–0.25, 2–8, 0.064–0.47, 0.75–32 μg/mL for *B. fragilis* strains isolated in Izmir. Of the Edinburgh strains 2, 2, 1 and 16 were determined to be resistant to clindamycin, amoxycillin clavulanate, cefoxitin, ciprofloxacin, respectively. Four strains were intermediate for ciprofloxacin. Of the Izmir strains 2 and 10 were detected to be resistant to clindamycin and ciprofloxacin, respectively. Two isolates were intermediate for both cefoxitin and ciprofloxacin. None of the isolates showed resistance to metronidazole and imipenem.
Conclusion: In conclusion, we can say that the resistance patterns of two hospitals were similar; metronidazole and imipenem are good empirical therapy choices of a suspected anerobic infection in these two hospitals while clindamycin, amoxycillin clavulanate and cefoxitin should be used carefully and ciprofloxacin does not seem to be an alternative drug for anerobic infections.

P1107 Comparison of susceptibility of *Bacteroides fragilis* group by MIC potency and pharmacokinetic-pharmacokinetic (PK-PD) profiles

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Objectives: The objectives were to compare in vitro potency of selected antimicrobials alone using in vitro parameters and to combine these values with PK-PD values to determine percentage of the dosing interval the drug level was over each MIC parameter (%T > MIC).
Methods: Over 400 Bfg clinical isolates were tested for susceptibility to piperacillin-tazobactam (PT), ampicillin-sulbactam (AS), imipenem (IM), meropenem (ME), and ertapenem (ER). MIC values (potency) were determined by NCCLS broth microdilution. Cumulative potency was compared using the mode MIC, MIC_{90}, and MIC_{99} values. Percent susceptibility (*S*) was determined using NCCLS breakpoints. PK-PD values from previous human trials using single or multiple dose area-under-curve values integrated with MIC parameters to determine percentage T > MIC (CID 27:10, 1998). A percentage T > MIC 50% was considered a positive endpoint.
Results: Percent *S* for PT, AS, IM, ME, and ER were 100, 89, 99.8, 99, and 99%, respectively. Mode MICs showed equal in vitro potency for P-T, IM, ME, and ER and was 8-fold > activity than AS. Calculated percentage T > MIC for PT (3.375 and 4.5 g dosing), IM (500 mg q 8 h), and ER (1 g q d) had comparable values of 115% whereas, AS (3 g of 6–8 h) had values 86–115%. MIC_{90} and MIC_{99}s indicated that IM was 4–8-fold more potent than ME and ER whereas PT was 4–8-fold more potent than AS. Using MIC_{90} and MIC_{99} values percentage T > MIC for PT and AS (3 g q 6) was 57–76 and 15–48%, respectively; for IM, ME, and ER the percentage T > MIC ranged from 71 to 96%, 42–92%, and 66–116%, respectively.
Conclusions: These data indicate variation in the in vitro potency of the broad-spectrum agents tested against the Bfg although using NCCLS breakpoints the susceptibility was virtually identical with the exception of AS. Using a predictive value of 50% integration of MIC parameters with PK/PD parameters percentage T > MIC values indicate dose dependent variation with PT, IM, ME, and ER showing positive predictive values against the Bfg isolates.

Bacterial vaccines

P1108 Proliferative responses in preimmunized mice lymphocytes by *Bordetella pertussis* outer membrane complex

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Objectives: *Bordetella pertussis* is the main etiologic agent of whooping cough. The nature of the immunity against *B. pertussis* infection and disease is poorly understood. Research on protection has traditionally been focused on the role of humoral pertssis specific antibodies. The aim of this study was to investigate pertussis cell mediated immunity in mice immunized with Outer membrane complex.
Methods: A group of mice immunized with OMC and OMP of *B. pertussis*. During a period of 7 weeks following the immunization, lymphocyte were isolated from lymph nodes of immunized mice. Cell proliferation was assayed by using 1E6 cells/mL in predetermined optimal dose of OMC and OMP. DNA synthesis was evaluated by counting H3 Thymidine incorporation.
Results: The blastogenic response of lymph nodes to 15 μg of OMP and OMC, and 10 μg of PHA was analyzed in preimmunized mice. At 30 days of postimmunization a significant increase in response to OMP and OMC was observed, but a small decrease in the level of response was evident against OMP and OMC at 60, 120 days of post immunization. But the response was still higher than observed at day 0.
Conclusion: Current findings evidently prove the capability of outer membrane complexes of *B. pertussis* in inducing the cell mediated immunity.

P1109 Pertussus toxin mimics effects of whole-cell pertussis vaccine upon hematopoiesis in mice

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Earlier we found that whole-cell pertussis vaccine (WPV) exerted complex and pronounced influence upon lympho-hematopoietic system in mice. Marked proliferation of hematopoietic stem cells (HSC) was observed in the bone marrow, followed by efflux of HSC from the bone marrow into peripheral blood. Also, a sharp rise in the numbers of endogenous spleen colonies was seen. Meanwhile, in thymus of WPV-treated mice an increase in numbers of suppressor T cells was found capable of suppressing antibody-forming cells activity as well as graft-versus-cell reaction, and proliferation of syngeneic HSC. Here we attempted to make a comparison of the effects mentioned above exerted by WPV in parallel with pertussus toxin (PT), or

lymphocytosis-promoting factor of *B. pertussis*—unique toxin responsible for most systemic reactions induced by WPV. PT isolated from *B. pertussis*, strain 475, was further purified using chromatography on hydroxilapatitis and lentilectin-Sepharose 4B columns, with phosphate buffer gradient and step elution at 4°C. PT consisted of four subunits with molecular weight 28.4, 24.3, 21.8, and 15.2 kDa, was found to possess specific lymphocytosis-promoting activity (0.2 μg per mouse increased leukocyte numbers 3-fold), and histamine-sensitizing activity. PT injected into CBA mice at a dose 0.2 μg/mouse was found to give a sharp rise in endogenous hematopoietic spleen colonies number in mice irradiated at a sublethal dose, to induce a pronounced proliferation of bone marrow HSC, revealed by hydroxyurea 'suicide' assay. It was also shown to induce a severe depletion of thymus cells, reaching its peak at day 4. Thymocytes obtained on day 2 or 3 after the injection of PT produced a suppressive effect on endogenous colony formation and GVHR. At day 1 following PT a sharp (20-fold) rise in HSC number was seen in the peripheral blood. Taken together, these data testify that PT mimics all effect of WPV upon lympho-hematopoietic system that were established earlier. As many of these reactions could be regarded as harmful side-effects, it means that the contents of PT in acellular pertussis vaccine preparations should be carefully controled.

P1110 Curently circulating *Bordetella pertussis* strains isolated from clinical material compared with strains in the DTP pertussis vaccine used in Poland since 1960

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Objectives: Although immunization with DTP in Poland has been continuously performed from 1960, an unexpected increase of pertussis cases has been notified in recent years. Different alleles of genes encoding S1 subunit of pertussis toxin (ptxS1) and pertactin (prn) in currently isolated strains in comparison with strains used in the locally produced DTP vaccine has previously been found. The aim of the study was to evaluate an influence of changes in pertussis vaccine strains compositions (VSC) in Poland taking place since 1960, in respect to sequences of ptxS1 and prn genes allelic variation seen currently. Additionally, a potential of locally produced DTP vaccine in elimination of strains harboring different genes alleles in animal model has been tested.
Methods: Alleles of ptxS1 and prn genes in VSC used for production within 1960–67, 1967–69, 1969–71, 1971–77, and 1978–2002 and in additional 20 currently isolated strains were determined by cycle sequencing. In animal model, groups of BALB/C mice (40 per vaccine and 40 per control) were immunized i.p. with 1/4 of DTP human dose twice in 2-week periods. After, mice were nasaly challenged with *B. pertussis* strains harboring ptxS1B/prn1, ptxS1A/prn1, and ptxS1A/prn2 allele combinations. The outcome of challenge was followed by performing CFU counts on lung homogenates from individual mice (5 per time point) sacrificed 2 h and 3, 7, 10, 14, 17, 20, 28 days after a challenge.
Results: Within 1960–1977, strains of ptxS1B/prn1, ptxS1D/prn1, and ptxS1A/prn1 allele combinations were used for vaccine production, and within 1978–2002, ptxS1B/prn1 was the only one combination found in the VSC. Two allele combinations: ptxS1A/prn1 (70%) and ptxS1A/prn2 (30%) were characteristic for strains isolated from pertussis cases within 1995–2001. Animal model study confirmed lower potential of DTP vaccination in elimination rates for strains harboring ptxS1A/prn2 and then pxS1A/prn1 in comparison with ptxS1B/prn1 allele combinations.
Conclusions: Our study indicates that possibility of vaccine immunity escape mutants appearance in Poland might took place in case of pertactin and not in case of pertussis toxin subunit S1, as not-vaccine variant (prn2) was not found in any VSC within decades and ptxS1A has been found in VSC within 1960–78. The results of the study suggest that a change in VSC in DTP in respect to prn profile might improve vaccine potential to eliminate currently circulating strains.

P1111 Persistent specific T-cell memory in adults after vaccination with acellular pertussis vaccine

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Objectives: In the absence of well-defined serologic correlates of protection in pertussis, the importance of cell-mediated immunity (CMI) has been acknowledged. We compared CMI responses in a subset of vaccinees in the NIH-funded Adult Acellular Pertussis Vaccine Efficacy Trial (APERT) and compared them with responses in control recipients.
Methods: 2781 subjects were randomized to receive either aP or control vaccine (hepatitis A). Lymphocytes from a subset ($n = 86$) enroled at one site were tested for pertussis-specific CMI responses before, 1 month and 1 year after vaccination. Proliferation and cytokine secretion (IFNγ and IL-5) from lymphocytes cultured in the presence of specific pertussis antigens (PT, FHA, PRN) were measured.
Results: One month and one year after vaccination the aP recipients ($n = 66$) showed significant increases in lymphocyte stimulation indices (LSI). One year after vaccination PT LSI decreased by 0.69-fold, but remained significantly higher than prevaccination levels. Control vaccinees ($n = 9$) showed no pertussis-specific CMI responses. Both IFNγ and IL-5 were secreted by pertussis antigen stimulated lymphocytes, with higher levels of IFNγ.

Table 1 Fold change in proliferation for aP vaccinees/controls ($N = 66/N = 9$) (comparison of mean SI)

Interval post vacc.	PT	FHA	PRN
Pre to 1 month post	4.75/1.21	5.18/1.36	2.95/0.97
1 month to 1 year	0.69/1.21	0.96/0.63	1.023/1.10

Conclusions: aP vaccines induced strong CMI response in adults that persisted for one year. Cytokine secretion patterns suggested a balanced Th1/Th2 immune response, comparable to those seen in animal models and in children after acellular pertussis vaccine. The duration of CMI responses should continue to be evaluated.

P1112 The possibility of using live-attenuated recombinant *Salmonella* strain as vaccine with rectal application

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Objectives: Chronic hepatitis B is associated with serious complications including cirrosis of the liver. T-cell mediated immunity removes infected hepatocytes. Activation of T-cell mediated immunity is provided HBc-ag. Thus, live-attenuated *Salmonella* expressing HBc-ag can be used as therapeutic vaccine.
Methods: Attenuated cya (lack of adenylatecyclase)-supressor (CRP) mutant of *Salmonella* s/t Enteritidis was used as vehicle for plasmid pkHBc expressing HBc-ag of Hepatitis B virus. The experimental suppositories for mice containing recombinant Salmonella in concentration 100 000 cells were prepared. Mice were single immunizied per rectum. Releasing of *Salmonella* with feces, its multiplication in spleen and ability to induce T-cell mediated immune response were examined.
Results and conclusions: The recombinant attenuated *Salmonella* strain did not release with feces and contaminate environment. The strain possessed ability to multiply in spleen after rectal inoculation during more then 35 days forming specific T-cell mediated immune response to HBc-ag. Thus, attenuated cya-supressive mutant of *Salmonella* s/t Enteritidis could be used as a deliver for HBc-ag in suppositories.

P1113 Immunologic evaluation of O-specific side chain of *Salmonella typhimurium* LPS conjugated with Tetanus toxoid

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Objectives: *Salmonella typhimurium* can cause serious infections in young children and in individuals with immunodeficencies. However, there is no vaccine against *S. typhimurium* licensed for human use. The O-specific polysaccharide (O-SP)of *S. typhimurium* is both a virulence factor and a protective antigen. Serum antibodies to the O-SP and phagocytosis of the bacteria are the main defense mechanisms against this bacterium. O-SP is not immunogenic enough, so in this study we conjugated purified O-SP with

tetanus toxoid (TT) and investigated its effects on induction of protective immune responses in mouse model.
Methods: O-SP was purified and conjugated with TT by carbodiimide-mediated amidation method. For obtaining of serum samples, BALB/c mice were injected with either conjugate, purified O-SP, or heat killed *S. typhimurium*, on days 0, 14 and 28. Animals were bled 2 weeks after last injection and serum samples were collected. Immunodiffusion was performed in agarose gel and standard ELISA method were used for measuring of specific antidodies against either O-SP or TT in serum samples. LD-50 was determined by the Reed and Muench method. Chemiluminescence was measured in a liquid scintillation spectrometer. In vitro phagocytic responses were studied by the Tomita method.
Results: High efficacy was realized with conjugation of the O-SP with TT. The immunodiffusion showed that conjugate reacted with both TT and *S. typhimurium* whole cell antiserums.O-SP did not elicit antibodies in mouse, however, either conjugate or heat killed *S. typhimurium* can elicit high levels of IgG in mouse after third injection. Efficient chemiluminescence emission was observed in the presence of either alive or heat killed *S. typhimurium* or O-SP-TT conjugate but not in the presence of O-SP alone, from mouse peritoneal macrophages. The mean number of ingested bacteria/cell in the presence of sera from immunized mice with purified O-SP, conjugate, heat killed bacteria and negative control were 6.96, 14.24, 15.96 and 6.67, respectively.
Conclusions: A conjugate that induces protective immune responses were developed and this conjugate also protects mice against challenge with pathogenic *S. typhimurium*.

P1114 Persistence of *Francisella tularensis* in the bone marrow of mice

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Studied were effects of live *Francisella tularensis* vaccine upon hematopoietic system in mice. Injection of bacteria at a dose 1 and 5 microbes per mouse induced a dose-dependent increase in the numbers of endogenous hematopoietic spleen colonies in mice irradiated at a sublethal dose. Further increase of inoculating number of bacteria to 10 microbe bodies per mouse resulted in a toxic death of irradiated mice. Also, a dose-dependent rise in proliferation of late bone marrow hematopoietic precursor cells (7-day CFUs) was observed using hydroxyurea 'suicide' technique. Proportion of CFUs in the S-phase of cell cycle increased 3–4-fold compared with controls. In order to study persistence of *F. tularensis* in the bone marrow of mice, a sensitive assay was developed enabling to reveal not only target cells for *F. tularensis* in hematopoietic tissues, but duration of the bacteria persistence in the bone marrow in the association with particular cells, as well. In brief, the assay involved incubation of bone marrow cells of infected mice either with specific rabbit anti *F. tularensis* antiserum that had previously been absorbed with normal bone marrow cells of syngeneic mice for 45 min at 37 °C, or with normal rabbit serum (absorbed as above to avoid possible cytotoxicity). The suspensions were then incubated at 4 °C for 60 min. At the end of that time 0.5 mL of fresh guinea pig complement (1:5) was added and the suspensions were further incubated 30 min at 37 °C. Then suspensions of both the antiserum-treated and control cells were injected i.v. into syngeneic mice irradiated 2 h previously at a lethal dose. Seven days later these recipients were sacrificed and their spleens removed and fixed in Bouin's solution. The spleen colonies formed by 7-day CFUs were counted, and a comparison between the cells treated with antiserum and those with normal serum made. Thus, we established that live *F. tularensis* are able to persist for at least 4 months in the bone marrow of mice in the direct association with 7-day CFUs. Other explanation could be that some antiserum-recognized antigens of the microbe was expressed at a surface of CFUs, thus making them susceptible to the lysis with complement. The proportion of CFUs affected in this manner fluctuated from 18 to 40%.

P1115 Production and characterization of monoclonal antibodies against *Brucella abortus* S(99) surface antigens

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Objectives: Considering the problems in specific and accurate diagnosis of Brucellosis, recently Monoclonal antibodies (MAb) have been used for identification and characterization of antigenic determinants of the *Brucella*.
Methods: In this research myeloma cells(Ag8.653) were fused with spleen cells of Balb/c mice immunized against *Brucella abortus* S (99) using polyethylene glycol. After screening by ELISA, hybridomas producing antibody were cloned by Limiting Dilution method. Double diffusion/Ouchterlony (DDO) method was used to identify the isotypes of produced MAbs. To determine the specificity of the anti-Brucella MAbs, Western immunoblotting was performed with cell extract (CE) of *B. abortus* S (99) separated by SDS–PAGE.
Results: In this study, six clones Ba-1, Ba-2, Ba-3, Ba-4, Ba-5 and Ba-6 producing antibody against *B. abortus* S(99) were obtained. MAbs of Ba-1, Ba-3, Ba-5 and Ba-6 were identified as IgG1 and Ba-2 and Ba-4 were identified as IgG2b. Only MAbs Ba-1 and Ba-2 cross reacted with *B. melitensis* 16 M and *B. suis*. Western blots of CE indicated that MAbs Ba-1, Ba-2 and Ba-3 mybe specific for S-LPS and Ba-4 and Ba-5 were reactive with major outer membrane protein (OMP) 25–27 kDa and Ba-6 was reactive with major OMP 36–38 kDa. All MAbs except Ba-6 have better reactivity with CE Ag.
Conclusion: With considering different patterns of the epitopes recognized by each antibody on the surface of bacterium, application of these antibodies in diagnosis, classification, epidemiology and immunology of this bacterium is considerable. The recognized bacterial antigens can be used as vaccine production and diagnostic agents.

P1116 Vaccine cold chain monitoring on the territory of the Republic of Serbia

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Objectives: Evaluation of vaccine cold chain quality during transportation and storage of DTP, OPV and BCG vaccines on the territory of the Republic of Serbia.
Methods: Monitoring was performed with the help of Vaccine Cold Chain Monitors (CCMs) made by Berlinger, Switzerland and Freeze Watch (FW) made by 3M, USA. Within the period of March–June 2002, 529 CCMs and 165 FW accompanied the procurement of DTP, OPV and BCG vaccines from the central warehouse in Institute Torlak, through all levels for vaccine supply up to the end users in vaccine points. On the territory of Serbia, monitoring encompassed 19 regions, including 86 vaccine points, which were chosen at random. The supervision of monitoring was done by the control of refrigerators in 40 vaccine points. Closed monitor cards were sent back to Institute Torlak, where the changes on CCMs and FW were analyzed.
Results: Up to the first level of supply of Regional Institutes for Public Health, 165 CCMs with FW were accompanied by 76180 doses of DTP vaccines, 196 CCMs were accompanied by 127220 doses of OPV and 168 CCMs were accompanied by 44800 doses of BCG vaccines. Out of this number, 128 (78%) monitor cards for DTP, 156 (80%) monitor cards for OPV vaccine and 123 (73%) monitor cards for BCG were sent back to the manufacturer. On the first level of supply, minimal exposure to temperature higher than allowed was registered in 50% CCMs for DTP, 40% for OPV and 45% for BCG vaccine. One percent of CCMs for OPV was exposed to the risk, that is, to temperature that endangers the vaccine. On the second level of supply, there was no exposure of vaccines to the risk. On the third level of supply, minimal exposure to temperature was registered in 84% CCMs for DTP, 73% for OPV and 100% for BCG vaccine. Exposure to the risk was registered in 2% CCMs for OPV vaccine. By the control of refrigerators during supervision visits, the high risk from freezing of vaccines wasn't noted, so that the high percent of FW cracking (64%), can be attributed to faults in monitors handling than to the real risk from freezing.
Conclusion: The cold chain plays the basic role in the success of immunization programme. These results will enable the correction of weaknesses that were registered in the cold chain system on the territory of Serbia.

P1117 Immunization safety—Serbian experience evaluation

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Aim: Quality evaluation of immunization practice.
Methods: Twenty-four immunization practice quality indicators were checked during supervision visits of 33, out of total 78, preschool children vaccination checkpoints, covered by the cold chain vaccine monitoring. During these visits, personnel (51 nurse and 37 physicians) voluntarily filled out the anonymous knowledge tests.

Results: Vaccine purchase evidence exists at 61% of checkpoints, temperature surveillance in refrigerators exists at 41% of checkpoints, and cold chain monitoring cards were correctly marked and positioned at 58% of checkpoints. Vaccine distribution in refrigerators and vaccine duration period were correct at 100% of checkpoints, while at 94% of checkpoints the opened vial policy was adequately conducted. Personnel accepted and now uses the AD syringes at 30% of checkpoints. Although a disposable syringes are exclusively being used for the immunization, all safe injecting procedures were observed only at 27% of checkpoints. The used material is being destroyed by incineration at 55% of checkpoints. On the question about the immunization needle recommended length, nurses gave correct answers in 39% of cases, there were 29% of incorrect answers, and 32% failed to answer. The correct application place for certain vaccines was marked in 46% of answers, and to the question on the cold chain vaccines there were 61% of correct answers. The most sufficient was the knowledge on the duration period of opened vials of DTP, OPV, MMR and BCG vaccines: 80% of answers were correct, 3% were incorrect, and 17% failed to answer. On the questions on contraindications, physicians answered: correctly in 68% of cases, incorrectly in 27% of cases, and 5% failed to answer. On the question on time-laps between the prescribed amounts of vaccines application: there were 45% of correct answers, 29% of incorrect answers and in 26% of cases the answer was omitted. By the education on the safe immunization strategy, at 66% of vaccination checkpoints, not one single member of the vaccination teams was encompassed.
Conclusion: The continuing education and training of the personnel which conducts immunization, along with the supervision of the adopted knowledge and skills, are the prerequisites for the safe immunization practice improvement in Serbia.

Respiratory tract infections

P1118 An evaluation of the best indicator set for predicting bacterial infection in patients presenting with an acute exacerbation of chronic bronchitis

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Objectives: In clinical trials of antibiotics, the identification of patients with an acute bacterial exacerbation of chronic bronchitis can be problematic as many exacerbations are not bacterial and this is difficult to ascertain in the initial consultation. This study aimes to examine the efficiency of: (i) patient symptoms (from Anthonison[1]); (ii) microscopic examination of sputum purulence; and (iii) Gram stain microscopy of organism types, in identifying patients with a bacterial infection.
Methods: Sputum samples were collected from patients with a history of chronic bronchitis, presenting with an acute exacerbation. Patients were required to have a worsening of at least two symptoms from: dyspnea, sputum volume and sputum purulence, and not have previously received an antibiotic for this episode. All centers were in Scotland and sputum samples were sent to the local accredited hospital laboratory for analysis (purulence by appearance and microscopy [>25WBCs and <10 epithelial cells per ×100 field], predominant cell types on Gram stain and culture). All patients gave informed consent.
Results: A total of 73 sputum samples were evaluated. There were 30 samples from males (41%) and 43 from females (59%). The mean age of the patients was 62.8 years (range 39–84 years). The four ineligible patients who were enrolled with one symptom were excluded from the analysis. The efficiency of symptoms, microscopic purulence and Gram stain in identifying patients with a bacterial infection are shown below.

	Total		Culture-positive		Culture-negative	
Criteria	No.	%	No.	%	No.	%
Sputum samples	73		43	58.9	30	41.1
All 3 symptoms	49	67.1	25	51.0	24	49.0
2 out of 3 symptoms	24	32.9	18	75.0	6	25.0
Microscopically purulent	34	46.6	25	73.5	9	26.5
Non-microscopically purulent	39	53.4	18	46.2	21	53.8
Organisms on Gram stain	66	90.4	41	62.1	25	37.9
No organisms on Gram stain	7	9.6	2	28.6	5	71.4
2 or 3 symptoms +microscopicaly purulent	34	46.6	25	73.5	9	26.5

Conclusion: There is no simple method of identifying patients with an acute exacerbation of chronic bronchitis and symptoms alone are a poor indicator of bacterial infection. For the purpose of clinical trials of antibiotics, a combination of two to three symptoms and microscopic confirmation of sputum purulence appears to be the best indicator of bacterial infection and can be expected to correctly identify bacterial infection in around 74% of patients.

Reference:
1. Anthonison NR *et al. Ann Int Med 1987; 106: 196–204.*

P1119 The character of infectious process in chronic obstructive pulmonary disease exacerbation

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Methods: 103 patients with COPD exacerbation were inspected, the age is 25–80 years old, mean age is 52.5 years old. The clinical inspection, spirografy, bronchofibroscopy, Gram bacterioscopy of sputum smears, quantitative bacteriological sputum research with definition of germ concentration in 1 mL of sputum, definition of *Chlamidia pneumoniae* and *Mycoplasma pneumoniae* antigens in the sputum by PCR method and IgG, IgM levels to them by immuno assay method were made. The reliability of differences was verified by Fisher exast test.
Results: In the whole patients group ($n = 103$) infectional character of COPD exaserbaton was reliable more often then noninfectional character (70 and 30% accordingly, $P < 0.01$); monoinfection was prevailed over mixt-infection (47 and 24% accordingly, $P < 0.01$), *Streptococcus pneumoniae* (18%), *M. pneumoniae* (14%), *C. pneumoniae* (6%), *Heamophilus influenzae* (5%), *Enterobacter* spp. (3%), *Moraxella catarrhalis* (1%) were revealed as main pathogens. *Mycoplasma pneumoniae* infection was more often revealed then *Chlamidia pneumoniae* infection as monoinfection (30 and 13% accordingly, $P < 0.05$), whereas *C. pneumoniae* infection was more often revealed as mixt-infection (44 and 4% accordingly, $P < 0.01$). Noninfectional character of COPD exacerbation was more often revealed in smokers and in patients with professional pollutants in anamnesis (39 and 14% accordingly, $P = 0007$; 41 and 13% accordingly, $P < 0.01$). Infectional character of COPD exacerbation in the form of *S. pneumoniae* monoinfection was revealed in non-smokers and in patients with absence of professional pollutants in anamnesis (78 and 18% accordingly, $P < 0.01$). We analyzed bronchial obstruction degree according to GOLD, WHO, 2001. At stage I of COPD (FEV < 80% of predicted, FEV1/FVC < 70%) infectional character of COPD exacerbation was reliable more often then noninfectional character (74 and 26% accordingly, $P = 0.03$), monoinfection was prevailed (51 and 22% accordingly, $P < 0.05$). At more high bronchial obstruction degree, the reliable differences between infectional and noninfectional character of COPD exacerbation rates and monoinfection and mixed-infection rates were not revealed.
Conclusion: In 70% of COPD patients, the exacerbations are associated with infection, whereas smoking and professional pollutants influence on COPD exacerbation etiology. The infectional process features in different stages of COPD are required futher investigations.

P1120 Importance of *Moraxella* (*Branhamella*) *catarrhalis* broncho-pulmonary infections in Iraqi elderly patients with underlying chronic cardio-pulmonary disorders

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Objectives: To assess rate of infection, clinical data of the patients, oropharyngeal carrier rate in elderly patients with underlying chronic cardio-pulmonary disorders, beta-lactamase production and antibiotic resistance patterns.

Methods: Study group, 300 adults having broncho-pulmonary infections. Carriers group, 200 randomly selected adults (79 controls and 121 with risk factors; having an underlying chronic cardio-pulmonary disorder, immunosuppressive factor and/or use antibiotics). The study period was from November 1995 to April 1996. Sputum samples of the study group selected by cellular criteria and Gram-stain directed culture method and Zieh–Nielsen stain. Isolated *B. catarrhalis* and other bacterial pathogens identified from microscopic and colonic features and proper differential tests. Swabs inoculated on *Branhamella* selective agar. Beta-lactamase production of *B. catarrhalis* isolates tested using rapid iodometric method. Single-disc diffusion method used for antibiograms.
Results: *Branhamella. catarrhalis* was isolated from 26/205 (12.68%), ranking third after *S. pneumoniae* and *Haemophilus* spp. in the criteria selected patients (205/300). Most affected patients were >50 years of age with significant difference from other pathogens (t of difference = 3.1447 and $P < 0.05$). The majority of affected patients had an underlying chronic cardio-pulmonary disorder (96.1%) more than other pathogens (Chi square = 4.856 and $P < 0.005$), and Beta-lactamase producing *B. catarrhalis* were found in 84% of them. The commonest presentation was acute exacerbation of underlying disorder (84.6%). Oropharyngeal carrier rate of B.catarrhalis in the study group adults was 4.5% (9/200), being higher in the risk (5.79%) than the control group (2.53%). Most *B. catarrhalis* isolates were beta-lactamase producers (29/35; 82.86%). All were resistant to penicillins.
Conclusions: It is important to include *B. catarrhalis* in the differential diagnosis of broncho-pulmonary infections in adults, especially in the elderly with an underlying chronic cardio-pulmonary disorder. Consider high rates of penicillin resistance in initial treatment.

P1121 Factors predicting bacterial etiology in acute exacerbation of COPD

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Background: Bacterial infection is a frequent cause of acute exacerbation in COPD (AECB). Risk factors for bacterial etiology were evaluated.
Methods: Outpatients and patients hospitalized for acute exacerbation of COPD were studied prospectively. Severity of exacerbation was rated according to Winnipeg criteria (I–III). The extent of underlying airflow-limitation was measured during stable disease, and was categorized according to the criteria of the ATS. Sputum samples were obtained from all patients prior to antibacterial therapy.
Results: Out of 272 individuals, 193 patients fulfilled the criteria of inclusion. In 114 subjects potential pathogenic bacteria were isolated, including *H. influenzae* ($n = 31$), *S. pneumoniae* ($n = 22$), *Moraxella catarrhalis* ($n = 19$), *P. aeruginosa* ($n = 12$) and Gram-negative enteric bacteria (GNEB) ($n = 19$). Univarate analysis identified severe airflow-limitation (FEV1 < 35% predicted), emphysema, hypercapnia, use of systemic steroids, a high lifetime dose in cigarette smoking, as well as prior antibacterial treatment as significant predictors of infection with GNEB and *P. aeruginosa*. Current smoking and the use of topic steroids were significantly associated with isolation of *H. influenzae*, *M. catarrhalis* and Gram-positive bacteria. In multivariate analysis severe airway-obstruction ($P = 0.017$) and the use of systemic steroids ($P = 0.017$) were independent predictors of infection with GNEB and *P. aeruginosa*. An absolute FEV1 of <1.1 L (sensitivity 85%, specificity 66%) or an FEV1 of <37.2% of the predicted (sensitivity 82%, specificity 74%) were the most accurate discriminators in the risk of infection with GNEB and *P. aeruginosa* as compared with *H. influenzae*, *M. catarrhalis* and Gram-positive bacteria.
Conclusions: *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* were the most frequent pathogens in bacterial infection in AECB. Several easyly accessible factors, however, predict infection with GNEB and *P. aeruginosa* and may therefore be helpful tools in directing antibiotic therapy in acute exacerbation.

P1122 Influence of levofloxacin compared with clarithromycin on the infection-free interval in patients with chronic bronchitis

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Background: Purulent exacerbation of chronic bronchitis in different severity stages is a continuous therapeutic challenge. The results of previous studies with fluorquinolones gave reasonable evidence for a prolonged time interval until the subsequent exacerbation episode.
Methods: In this randomized, multicentre, double-blind clinical study, levofloxacin (LFX) was compared with claritromycin (CLA) in patients with acute bacterial exacerbation of chronic bronchitis (ABECB) graded GOLD II and Winnipeg I/II. The main outcome variable for establishing clinical efficacy was the time interval between two consecutive episodes of exacerbation within an observation period of one year. The patients were randomly allocated to either receiving LFX (500 mg od for 7 days) or CLA (250 mg bid, for 10 days). A total of 504 patients (female 45%, male 55%, mean age 59.7 ± 11.1 years) with mean FEV1 of 58.1–12.1% measured in the infection-free interval prior to the acute episode were included in the intention-to-treat analysis. Demographic and baseline characteristics were comparable amongst both groups.
Results: Satisfactory clinical outcome was observed in 82.8% of the LFX-group and 79.6% of the CLA-group ($P = 0.474$). However, the microbiological eradication rates were higher ($P < 0.001$) for the LFX-group (96.0%) than for the CLA-group (81.9%). Patients with a confirmed bacterial exacerbation before treatment could be evaluated for the main objective. The mean infection-free interval was 117 days for the LFX-group compared with 107 days for the CLA-group ($P = 0.378$). Due to the low number of patients with proven bacterial etiology ($n = 100$) significance level could not be reached.
Conclusion: The treatment of ABECB with 7 days LFX (od) revealed a tendency towards better clinical results than with 10 days CLA (bid), although not statistically significant. However, the bacterial eradication rate was significantly higher for the LFX-group and a meaningful trend to a longer infection-free interval could be observed.

P1123 Levofloxacin vs. ceftriaxone/azithromycin in the treatment of community-acquired pneumonia: a length of stay comparison

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Objectives: Recently, there has been increased use of the fluoroquinolone, levofloxacin as first line treatment in community-acquired pneumonia (CAP), possibly because of its lower acquisition cost relative to other recommended regimens such as a cephalosporin/macrolide combination. Currently, there are no data analyzing hospital length of stay (LOS) in patients treated with levofloxacin vs. other regimens, despite the fact that LOS can significantly impact the total cost of CAP treatment. This retrospective study was designed to compare LOS data for CAP patients treated with single-agent levofloxacin vs. those treated with ceftriaxone/azithromycin (CX/AZ).
Methods: In a retrospective chart review conducted at two community teaching hospitals, inpatient data from 434 CAP patients were reviewed for LOS, clinical outcomes and need for additional antibiotic therapy. Patients treated with levofloxacin were carefully matched to patients receiving CX/AZ or other single-agent antibiotic therapy according to Fine risk classification. The primary comparison was length of stay.
Results: At baseline, mean Fine scores were lower (indicating lower severity) in the levofloxacin group (91) compared with the CX/AZ group (98) ($P < 0.001$). Despite this, mean LOS was longer in the levofloxacin group (6.8 days) than in the CX/AZ group (4.4 days) ($P < 0.001$). Nineteen patients in the levofloxacin group had a LOS > 13 days; a separate analysis excluding these patients produced similar results. Risk class by itself also had a significant effect on LOS ($P < 0.001$). In the levofloxacin group, 36% of patients required additional antibiotics, compared with 8% of CX/AZ patients.
Conclusions: Despite lower Fine risk scores, levofloxacin was associated with longer hospital stays and more supplemental antibiotic usage compared with CX/AZ, both factors that could translate into significant cost savings for the hospital. Treatment cost/benefit analysis must take into account total clinical outcome with first line therapy rather than simply the pricing of an individual drug.

P1124 Levofloxacin in the treatment of community-acquired bacteremic pneumococcal pneumonia

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Objectives: To report on the cumulative clinical trial experience of levofloxacin in the treatment of patients with community-acquired pneumonia (CAP)-related pneumococcal bacteremia.
Methods: Retrospective review of all Robert Wood Johnson Pharmaceutical Research Institute/Ortho-McNeil Pharmaceutical trials to identify those

patients with CAP-associated pneumococcal bacteremia. Results available to-date reflect the efficacy of the 500 mg i.v. and/or PO once daily for 7–14 days regimen. Data from recently completed CAP trials utilizing a regimen of 750 mg i.v. and/or PO once daily for 5 days will also be included along with calculated drug exposure rates based on the kinetics of the two doses and recent national surveillance data.

Results (data available to-date): Ninety-five microbiologically evaluable patients were treated with levofloxacin for CAP who had at least one blood culture positive for *Streptococcus pneumoniae* (SP). Eleven cases were due to penicillin nonsusceptible strains of SP: five with high-level (MIC ≥ 2 μg/mL) and six with intermediate-level penicillin resistance (MIC $= 0.1$ μg/mL). Thirteen percent (11/82) of the tested bacteremic isolates were macrolide-resistant. Ninety-two (96.8%) PB patients were considered clinical successes (including all cases due to penicillin nonsusceptible SP) at the test-of-cure evaluation; 91 (95.8%) SP isolates were eradicated or presumed eradicated.

Conclusion: Levofloxacin is effective in the treatment of CAP-associated pneumococcal bacteremia, including disease due to penicillin- and macrolide-resistant strains.

P1125 First postmarketing data on treatment of community-acquired pneumonia with moxifloxacin i.v. – an interim analysis

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Objectives: This postmarketing surveillance study is being conducted to investigate the efficacy and tolerability of moxifloxacin i.v. therapy in community acquired pneumonia under general hospital conditions.

Methods: This open, prospective, noncontrolled, nonrandomized multicenter study is currently on-going in German hospitals. Patients with documented community acquired pneumonia (CAP) treated with moxifloxacin (MXF) i.v. or with MXF sequential therapy (i.v. and switch to oral) are enrolled. The exclusion criteria are the contraindications mentioned in the summary of product characteristics. Documentation includes demography, anamnesis, prior antibiotic treatment, concomitant diseases and medications, daily recording of MXF therapy and symptom status, overall assessment of therapy with moxifloxacin i.v. and reporting of all adverse events observed within the treatment period.

Results: 308 patients with CAP treated with 400 mg MXF once daily were documented until January 2003, 52.6% males and 47.4% females, mean age 65.6 (SD = 16.2) years. Duration of MXF therapy was up to 4 days in 10.4% of patients, 5–7 days in 49.4%, 8–10 days in 29.9% and >10 days in 10.4% of patients. In case of sequential therapy (80.5% of patients), MXF i.v. was given for 1 day in 11.3% of patients, 2 days in 26.2%, 3 days in 29.4%, 4 days in 18.2% and more than 4 days in 14.9% of patients. Improvement of clinical symptoms is shown in Fig. 1. Improvement was reported in 59.1% of patients at day 3, 85.4% at day 5 and 90.6% at day 7. An overall improvement of CAP was reached after a mean of 3.2 days, cure after a mean of 6.7 days. Efficacy of MXF i.v. therapy was rated by the physicians as very good or good in 87.6% of all cases. Tolerability was rated in 97.7% of patients as very good or good. For 18 patients (5.8%) adverse events were recorded, but only in two of these patients (0.65%) events were considered related to the MXF therapy.

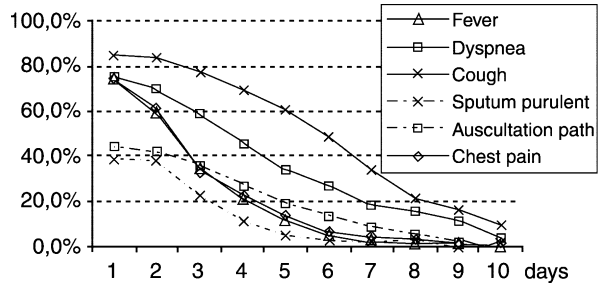

Figure 1 Improvement of clinical symptoms over 10 days of MXF treatment.

Conclusions: The new moxifloxacin i.v. formulation is safe and highly effective in general hospital practice. Due to the rapid symptom relief, Moxifloxacin allows for an early change from the i.v. to oral application in patients with CAP treated in hospital.

P1126 Daily practice treatment of bacterial respiratory tract infections with moxifloxacin

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Objectives: This postmarketing surveillance study was conducted to evaluate efficacy and tolerability of moxifloxacin (MXF) in the treatment of bacterial respiratory tract infections under daily-life conditions.

Methods: This open, prospective, noncontrolled, multicenter study was carried out in general practice settings throughout Germany. Patients with documented acute exacerbation of chronic bronchitis (AECB), community-acquired pneumonia (CAP) or acute sinusitis were enrolled. Exclusion criteria were the contraindications mentioned in the summary of product characteristics. Documentation included demography, prior antibiotic treatment, concomitant diseases and medication, onset of symptoms, duration and overall assessment of the MXF therapy and reported adverse events.

Results: A total of 9036 patients (4328 AECB, 1467 CAP, 2405 sinusitis, 836 other) treated with 400 mg MXF once daily were documented by 1575 physicians. Gender distribution was comparable with a mean age of 50.8 ± 17.0 years. Duration of MXF therapy was 5 days for 42.4% of patients, 7 days for 38.9%, 10 days for 17.5%, less than 5 days for 0.9% and more than 10 days for 0.2%. Clinical cure rates for single symptoms (all indications) were 59.3–96.7% at the last visit. The results of the three different diagnoses are shown in Fig. 1. Overall improvement rate after 3 days was 54.2% for CAP, 61.8% for AECB and 71.6% for sinusitis. After 7 days, these rates were 97.1, 97.4, and 99.0%, respectively. The assessment 'very good' or 'good' was given by the physicians for overall efficacy of MXF therapy in 95.4%, for overall tolerability in 96.8%, and for patient acceptance in 97.2% of all cases. In 72 patients (0.8%), there were adverse drug reactions recorded, the majority being gastrointestinal disorders followed by nervous system disorders.

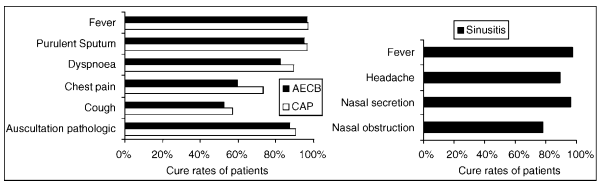

Figure 1 Cure rates of single symptoms at least visit for the different diagnoses AECB, CAP and sinusitis.

Conclusions: MXF used under normal daily practice conditions has excellent efficacy and safety and should be considered to be a first line antibiotic in the treatment of AECB, CAP and acute sinusitis.

P1127 Telithromycin is highly effective against macrolide-resistant *Streptococcus pneumoniae*: a pooled analysis of 11 multicenter clinical trials

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Objectives: The increasing worldwide prevalence of macrolide (erythromycin A [ERY]) resistance among *Streptococcus pneumoniae* may complicate the choice of treatment regimen for respiratory tract infections (RTIs) including community-acquired pneumonia (CAP) and acute maxillary sinusitis (AMS). Telithromycin (TEL), the first ketolide to be approved for clinical use, demonstrates excellent rates of clinical and bacteriological efficacy in the treatment of RTIs caused by common bacterial pathogens, including resistant strains, as well as atypical/intracellular respiratory pathogens. We report here the efficacy of TEL in patients with CAP or AMS caused by strains of *S. pneumoniae* resistant to ERY (ERSP).

Methods: Pathogens causative for infection were isolated from adult patients enrolled in one of 11 multicenter studies and who had received 5–10 days' treatment with TEL 800 mg once daily. Clinical and bacteriological outcomes were determined at the post-therapy/test-of-cure visit (Days 17–21). A total of 409 isolates of *S. pneumoniae* (318 from CAP patients and 91 from AMS patients) were tested for susceptibility to ERY at a central laboratory using NCCLS broth microdilution procedures. Resistant strains (MIC ≥ 1 mg/L) were genotyped by PCR.

Results: In the TEL-treated per-protocol population, ERSP were isolated from 50/409 (12.2%) patients. Clinical cure and bacteriological eradication (documented or presumed) rates were high (both 86.0% [43/50]), and comparable with the rates obtained for the overall patient population with pneumococcal isolates (clinical cure, 93.4% [382/409]; bacteriological eradication, 94.9% [388/409]). Isolates from 29 patients with ERSP had ERY MICs $\geq$ 16 mg/L at baseline, and 26 (89.7%) of these patients, including 9/9 (100%) with MICs of 512 mg/L, achieved clinical cure with bacteriological eradication following treatment with TEL. The majority of ERSP isolates with MICs $<$ 16 mg/L were genotyped as mef(E) positive (17/21), whereas for those with ERY MICs $\geq$ 16 mg/L, 19/29 were erm(B) positive. TEL was well tolerated with the majority of treatment-emergent adverse events (TEAEs) reported as mild to moderate and self-limiting. The most common TEAEs were diarrhea, nausea and headache.
Conclusions: TEL displays high clinical and bacteriological efficacy against ERSP isolates from patients with CAP or AMS, including those with elevated MICs to ERY, irrespective of the resistance genotype.

P1128 Potent activity of telithromycin in the treatment of key respiratory pathogens associated with community-acquired upper and lower respiratory tract infections

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Objectives: To evaluate the clinical and bacteriological efficacy of telithromycin (TEL) in the treatment of key bacterial pathogens associated with community-acquired RTIs.
Methods: Data were pooled from 14 Phase III/IV studies of TEL in the treatment of community-acquired pneumonia, acute exacerbation of chronic bronchitis and acute sinusitis. Key causative RTI pathogens (*Streptococcus pneumoniae*, *Haemophilus influenzae, Moraxella catarrhalis* and *Staphylococcus aureus*) were isolated at entry from respiratory and blood samples and tested for their *in vitro* susceptibility to TEL, penicillin G (PEN) and erythromycin A (ERY). Clinical and bacteriological efficacy of TEL at the post-therapy/test-of-cure visit (Days 17–24) was examined in patients with a microbiologically evaluable pathogen isolated at entry.
Results: The majority (99.4%, 1530/1540) of key RTI pathogens identified from the bacteriologically evaluable modified intent-to-treat population (bmITT), including PEN- and ERY-resistant strains of *S. pneumoniae* (PRSP and ERSP, respectively), were inhibited by TEL: 1466/1540 strains were inhibited at TEL concentrations of $\leq$1.0 mg/L (streptococci and staphylococci), $\leq$4.0 mg/L (*H. influenzae*) or $\leq$0.12 mg/L (*M. catarrhalis*), and 64/1540 strains at TEL concentrations of 2.0 mg/L (streptococci and staphylococci), 8.0 mg/L (*H. influenzae*) or $\leq$1.0 mg/L (*M. catarrhalis*). Treatment with TEL 800 mg once daily for 5, 7 or 7–10 days resulted in high overall rates of clinical cure (88.1%; 1593/1808) and satisfactory bacteriological outcome (89.0%; 1593/1789), similar to those seen with comparator antibacterials (clinically evaluable bmITT). Excellent clinical cure and bacterial eradication rates were achieved for the following key RTI pathogens: *S. pneumoniae* 92.7, 94.3%; PRSP 82.3, 82.3%; ERSP 84.6, 84.6%; *H. influenzae* 87.0, 85.6%; *M. catarrhalis* 89.7%, 89.7%; *S. aureus* 84.9, 87.7%, respectively (clinically evaluable per-protocol populations). TEL was well tolerated, diarrhoea, nausea and headache were the most frequent adverse events, and the majority were of mild-to-moderate intensity.
Conclusion: TEL provides potent activity against key RTI pathogens, including drug-resistant *S. pneumoniae*, with high rates of clinical cure and bacterial eradication. This targeted spectrum of activity, combined with a short treatment regimen, makes TEL an effective option for the first-line therapy of community-acquired RTIs.

P1129 Five- to 10-day therapy with telithromycin is highly effective in outpatients with pneumococcal bacteremia associated with community-acquired pneumonia

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Objectives: The progression of community-acquired pneumonia (CAP) to bacteremia is one of the most serious complications of this disease, and has been associated with an increased risk of mortality. The objectives of this analysis were to determine the clinical and bacteriological efficacy of telithromycin (TEL) — the first ketolide antibacterial – in the treatment of outpatients with pneumococcal bacteremia associated with CAP.
Methods: A total of 2991 patients ($\geq$13 years) with radiologically confirmed CAP participated in eight Phase III/IV multicenter studies (four comparative and four open-label studies). Overall, 2289 patients were treated with TEL 800 mg once daily for 5–10 days (one study comprised 5- or 7-day TEL), and 702 received a comparator antibacterial for 7–10 days (amoxicillin, clarithromycin or trovafloxacin). The incidence of pneumococcal bacteremia was determined from blood samples taken upon study entry. Clinical and bacteriological outcomes were determined at the post-therapy/test-of-cure (TOC) visit (Days 17–21).
Results: Of the 2289 TEL-treated patients, 94 had documented pneumococcal bacteremia, and 82 of these patients were evaluable in the per-protocol (PP) population. Infection severity at baseline was comparable between the TEL-treated CAP patients and the subset of patients with bacteremia (15.5% and 17.0% had Fine scores $\geq$III, respectively). Clinical cure rates for the TEL-treated patients with bacteremia were high (74/82 [90.2%]), and comparable with the total CAP PP population (1755/1925 [91.2%]). Bacteriological eradication (documented or presumed) of *S. pneumoniae* was achieved in 77/82 (93.9%) TEL-treated bacteremic patients vs. 305/318 (95.9%) patients in the overall CAP population. The clinical and bacteriological efficacy observed with pooled comparator antibacterials in bacteremic patients was 15/19 (78.9%). Clinical cure was also high among TEL-treated patients infected with strains of *S. pneumoniae* resistant to either penicillin G (5/7), or erythromycin A (8/10). TEL treatment was well tolerated; the majority of treatment-emergent adverse events were of mild to moderate intensity, the most common of these being diarrhea, nausea, headache and vomiting.
Conclusions: Oral TEL therapy is an effective, first-line treatment for patients with mild to moderate CAP, including those with pneumococcal bacteremia, providing high levels of clinical cure and bacteriological efficacy.

P1130 A 4-year prospective microbiological surveillance study of confirmed respiratory infection in a university hospital in northern Italy

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Objective: To assess the frequency and microbiological features of lower respiratory tract infection in patients followed at a tertiary care hospital in Italy.
Methods: Only reliable specimens (tracheobronchial aspirate (TBA), bronchoalveolar lavage fluid (BAL), and bronchial brushing (BB)), submitted for microbiological evaluation were prospectively considered, 1999 to June 2002.
Results: In the 42-month study period, 8376 specimens were submitted, mostly represented by TBA (7623 : 91%), followed by BAL (698), and BB (55 cases). The great majority of samples came from intensive care units (ICU), which accounted for 6649 TBA of 7623 (87.2%), 353 BAL of 698 (50.6%), and 29 BB of 55 (52.7%). Medical departments (D) accounted for 772 specimens, surgical D for 218, pediatric D for 124, cardiology D for 114 samples, and hematology D for 33. When evaluating the temporal trend of submission, 2543 samples were examined in the year 2000, followed by 2001 (2291), and 1999 (2156). When considering overall respiratory secretions, a positive yield was obtained in 3277 materials of 8476 (38.7%), with higher frequency for BAL (44.6%), followed by TBA (38.8%),and BB (16.4%), in absence of significant variations in the 4-year study period, but a peak BAL positivity rate in the year 2001 (57.3%). Focusing on specimens coming from ICU, TBA had a positivity index ranging from 35.1% (year 2002) to 40.5% (year 1999), BAL positivity varied from 46.9% in 1999 to 65.6% in 2001, while BB proved positive in 11.1% of cases in 2001, up to 33.3% in 2002. Among the 26 different identified organisms, *Pseudomonas aeruginosa* ranked first (1176 isolations on the whole, 372 in the year 1999), followed by *S. aureus* (1103 episodes, 379 in the year 1999), and *Candida albicans* (1009 312 in the year 2001), regardless of submitted material. All other 23 bacterial and fungal organisms were isolated in $<$10% of cases, with *Stenotrophomonas maltophilia, Serratia marcescens, Alcaligenes xylosoxidans*, *Klebsiella pneumoniae*, *Escherichia coli*, and Enterobacter cloacae as the most frequent bacteria. *Mycobacterium tuberculosis* was isolated in 37 cases (24 from BAL,13 from TBA), and *Candida* spp. was the only organism who had a significantly increased yield over time ($P < 0.01$ in 2002 vs. 1999).
Conclusion: A permanent monitoring of microbiological features of the most common infectious diseases, such as confirmed lower respiratory tract

infection, is a cornerstone for understanding their epidemiology, and planning diagnostic, therapeutic, and prophylactic measures.

P1131 Comparative antibacterial activity of 12 antibiotics against invasive *Streptococcus pneumoniae* strains, isolated in Romania from January 1996 to September 2002

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Objectives: to study the antibiotic resistance in pneumococci isolated in Romania between January 1996 and September 2002.
Methods: The strains of *S. pneumoniae* collected at the National Reference Center for Streptococcus between January 1996 to September 2002 ($N = 1217$) coming from sputum or tracheal aspirate ($N = 570$), blood and CSF ($N = 492$) and others ($N = 155$): ear and eye fluid, sinus, synovial fluid were serotyped and tested for susceptibility (MICs) to 12 antibiotics. The isolates were analyzed for susceptibility to the following antibiotics:penicillin (Pc), erythromycin (Em), cephalothin (Kf), cefuroxim (Cef), cefotaxim (Ctx), cotrimoxazole (Sxt), chloramphenicol (Cm), ofloxacin (Ofx), amoxicillin (Amx), amoxicillin/clavulanic acid (Amc), tetracycline (Te), vancomycin (Va) by standard agar dilution MIC testing.
Results: Interpretative criteria were used according to NCCLS 1999. During the study period, penicillin-resistant strains of *S. pneumoniae* were noted as follows: 40% (MIC 50 : 0.06 mg/L, MIC 90 : 2 mg/L) in sputum and tracheal aspirate, in blood and CSF 28% (MIC 50 : 0.015 mg/L, MIC 90 : 0.5 mg/L) and in others 67% (MIC 50 : 0.5 mg/L, MIC 90 : 4 mg/L). During the study period the resistance revealed the following aspects:a) strains from sputum and tracheal aspirate: Em 28%, Te 28%, Kf 35%, Cef 20%, Ctx 4%, Amx 11%, Sxt 25%, Ofx 1%; (b) strains from blood and CSF: Em 10%, Te 24%, Kf 24%, Cef 18%, Ctx 12%, Amx 10%, Sxt 16%; (c) other strains: Em 54%, Te 39%, Kf 51%, Cef 39%, Ctx 18%, Amx 27%, Sxt 62%, Ofx4%. No resistant strain to vancomycin was found. The following serotypes were resistant to penicillin: 23 (34%), 6 (29.7%), 19 (25.3%), 14 (11%).
Conclusions: An alarming increase of the resistant pneumococci to Pc, Em, Sxt, and Kf was noted in the last years in Romania. There is an urgent need to implement a network in Romania for the surveillance, prevention and control of antibiotic-resistant pneumococci and antimicrobial usage in hospitals and to enhance the use of an efficient pneumococcal vaccine.

P1132 Antimicrobial resistance patterns of respiratory pathogens: a local report from Turkey

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Objectives: Commonly used antimicrobials for respiratory tract infections (RTI) were tested against 142 *Streptococcus pneumoniae*, 79 *Haemophilus influenzae* and 18 *Moraxella catarrhalis* strains isolated from patients with community acquired infections.
Methods: Strains were isolated both from adult ($n = 104$) and children ($n = 135$) patients between 1999 and 2000. The specimens included sputum ($n = 123$), bronchioalveoler lavage ($n = 38$), blood ($n = 26$), deep tracheal aspirate ($n = 17$), cerebrospinal fluid ($n = 3$), ear aspirate ($n = 11$) and conjunctival smears ($n = 21$). Antimicrobial susceptibility testing was performed by Etest strips (AB Biodisk, Solna, Sweden) and the results were evaluated according to the NCCLS criteria.
Results: Intermediate-level penicillin resistance was observed in 38.7% of *S. pneumoniae* isolates and there was only one isolate being highly resistant to penicillin. Resistance to clarithromycin and trimethoprim-sulfamethoxazole was 13.3% and 64.0%, respectively. All pneumococci were susceptible to ceftriaxone and levofloxacin. Of the *H. influenzae* strains tested, 3.8% were beta-lactamase positive whereas resistance to clarithromycin and trimethoprim-sulfamethoxazole was 7.5% and 31.6%, respectively. Of the 18 *M. catarrhalis* isolates tested, 8 of them were producing beta-lactamase. There was no resistance against ampicillin-sulbactam, cefuroxime, ceftriaxone, ciprofloxacin and levofloxacin among *H. influenzae* and *M. catarrhalis* strains.
Conclusion: As several factors may have an impact on the pattern of resistance, there might be geographic variation in antimicrobial resistance. Therefore, local/national surveillance data is of crucial value for empiric treatment.

P1133 Antimicrobial resistance among respiratory tract pathogens of *Streptococcus pneumoniae* and *Haemophilus* influenzae in Sao Paulo Brazil from 1996 to 2000

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Westlake, USA; London, UK; Cleveland, USA; Sao Paulo, BR

Background: As part of the Alexander Project, this study was undertaken to assess the *in vitro* activity of several antimicrobial agents against *Streptococcus pneumoniae* and *Haemophilus influenzae* from Sao Paolo Brazil in 1996–2000, including penicillin, amoxicillin/clavulanic acid (A/C), ampicillin, amoxicillin, cefaclor, cefdinir, cefixime, cefprozil, ceftriaxone, cefuroxime, azithromycin, clarithromycin, erythromycin, ciprofloxacin, levofloxacin, ofloxacin, chloramphenicol, clindamycin, doxycycline, and trimethoprim/sulfamethoxazole (T/S).
Methods: MICs were determined by the National Committee for Clinical Laboratory Standards (NCCLS) method by a central laboratory and interpreted with NCCLS and PK/PD breakpoints.
Results: Overall, 79.0% of *S. pneumoniae* were penicillin susceptible, 19.0% intermediate and 2.0% resistant and rates were fairly consistent through out the five years of testing (ranging from 69.7 to 85.9% susceptible, 14.1–29.0% intermediate and 0–3.4% resistant). The most active agents against *S. pneumoniae* were amoxicillin, A/C, ceftriaxone and levofloxacin; T/S was the least active agent (the percentage of nonsusceptible strains ranged from a low of 35.8% in 1996 to 78.9% in 1999). Macrolide, chloramphenicol and clindamycin resistance rates were less than 6%. The prevalence of doxycycline resistance among *S. pneumoniae* was 26.4%. β-lactamase was produced by 13.7% of *H. influenzae.* One hunhred per cent were susceptible to A/C, cefdinir, cefixime, ceftriaxone, and quinolones. The prevalence of chloramphenicol resistance among *H. influenzae* was 8.6%. The least active agents against *H. influenzae* were T/S (the percentage of nonsusceptible strains ranged from 27.0% in 1996 to 73.2% in 1999) and the macrolides (overall less than 2.6% were susceptible to azithromycin based on PK/PD breakpoints).
Conclusions: With the exception of trimethoprim/sulfamethoxazole against both pathogens and chloramphenicol vs. *H. influenzae*, the prevalence of resistance in Sao Paulo remains relatively low and stable in comparison to other parts of the world.

P1134 Improving the management of community-acquired pneumonia: barriers to high-quality care in a UK teaching hospital

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Objectives: Community-acquired pneumonia (CAP) requiring hospital care is associated with considerable mortality (30-day = 19%) and resource use (median hospital stay = 7 days) in Tayside, UK. We have previously identified suboptimal processes of care for CAP [Clin Infect Dis 2001; 32 : 728–41]. As part of an ongoing quality improvement programme, we have investigated why CAP is suboptimally managed by combining quantitative and qualitative methodology.
Methods:
1. Structured survey of hospital doctors ($n = 83$) using ordinal scales (e.g. 1 = very poor, 5 = excellent), open questions and clinical scenarios analysis.
2. Semi-structured in-depth interviews ($n = 8$) analyzed using 'framework' methodology.
3. Field-note analysis of patients ($n = 22$) receiving delayed (>4-h post-admission) and/or inappropriate antibiotics.

Results:
1. Structured survey: previous training experiences (median = three out of five), self-reported familiarity with the British Thoracic Society (BTS) guideline (median = 1) and the acute medical admissions ward environment (median = 3) were found to be potential barriers to high-quality CAP care. Only 4% could correctly state the four core BTS severity criteria and 43% could not state a BTS adherent antibiotic regimen for severe CAP.
2. Barriers emerging from in-depth interviews include: previous CAP training, clinical experience, awareness/familiarity with guidance, difficulty in diagnosing CAP, confidence and the perception of ones ability to manage CAP, work intensity, ward organisation, staff communication, priority status of CAP and guideline usability. Important relationships between themes were: (a) the link between clinical experience/knowl-

edge, clinician confidence/perception and the likelihood of using available guidance and (b) high work intensity and low priority status of CAP resulting in delayed diagnosis and management.
3. Six barriers (occurring on 34 occasions) were identified in the 22 patients who received delayed and/or inappropriate antibiotic therapy. The three most important were: (a) failure to prescribe a one-off ('stat') dose (50%); (b) inappropriate antibiotics according to severity assessment (41%); and (c) wrong initial diagnosis (27%).

Conclusions: The combined results demonstrate the complex and multiple barriers to high-quality CAP care that exist in a UK teaching hospital. Improving the process (quality) of CAP care is likely to require a multifaceted intervention addressing these barriers.

P1135 Rising incidence of Legionnaire's disease in Switzerland from '99 to '02

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Objectives: Determination of the incidence of LD in CH from 1999 to 2002 by means of an epidemiological surveillance system originally instituted in 1987

Methods: LD is notifiable in CH. In response to mandatory notifications (Ns) by laboratories (positive cultures, IFA, DFA, urinary antigen (UA)), physicians are legally required to fill in a questionnaire (Q) on every patient. Laboratory Ns and Qs are entered into a data base on a case by case basis. Case definitions of the European Working Group for Legionella Infections (EWGLI) apply, and confirmed and probable cases are evaluated, analyzed routinely, and tabulated. Ns are updated weekly on the internet (http://www.bag.admin.ch) and include preliminary unconfirmed reports.

Results: Between 1999 and 2001 the SFOPH received 302 Ns of which 261 were retained while 25 possible and 16 doubtful cases were excluded. The case fatality rate was 10%. Most cases were sporadic. No major outbreak was identified. A total of 211 cases were UA-positive (serogroup (sg) unknown; specific kits for sg 1 are most used in (CH). Only 34 cases were confirmed by culture (more than 80% *Legionella pneumophila* sg 1). Nosocomial infections made up 10%, LD due to professional exposure 2%, and travel-associated LD 20% of all 261 confirmed and probable cases of LD. The median delay between Ns and the Qs was 4 weeks. The 180 preliminary Ns received to date for 2002 represent a more than twofold increase from 1999 to 2002 which is of concern.

Conclusions: The annual incidence in CH of about two notified cases of LD per 100 000 population is among the highest in Europe despite high sanitary technical standards and good implementation thereof. Guidelines published in 1999 and media attention to domestic cases and outbreaks abroad have resulted in increased public and physician awareness of LD, possibly leading to improved reporting. The scarcity of positive cultures which parallels the growing use of the UA test renders the tracking and linking of cases and their environmental source(s) increasingly difficult. The role of lowering hot water temperatures (to save energy) in the rising incidence of notified LD remains conjectural. The reporting system is presently too slow to detect outbreaks in time for appropriate preventive interventions.

P1136 *Legionella pneumophila* disease in Bilbao, Spain

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Objectives: The aim of this study was to know the epidemiological and clinical features of *L. pneumophila* in our hospital.

Methods: The study was a retrospective clinical investigation. We reviewed the clinical records of all patients with urine *L. pneumophila* antigen detection test positive (Binax Now/μ) and/or a culture positive at the Basurto Hospital during the period 10/1998–10/2002.

Results: During the 4-year study period were diagnosticated 73 patients with *L. pneumophila* pneumonia: 3 HIV positive (4.1%) and 70 HIV negative patients with male predominance 4/1and a mean age 57.31 (range, 25–74 years). There were 29 patients older than 65 years (39.7%). The distribution was along the year but there were 52 patients hospitalized during August–November. The predisposing factors were an elevated intake of alcohol 28 (45.2%), history of smoking 33 (45.2%) and chronic diseases in 30 (41.1%). Two were receiving inmunossupresor treatment. High fever (86.3%) was a main symptom, also were headache 19 (26%), and myalgia 15 (20.5%). The respiratory symptoms were cough nonproductive 14 (19.2%), cough and expectoration 22 (30.1%), dyspnea 9 (12.3%), chills 12 (16.4%) and chest pain 11 (15.1%). Gastrointestinal symptoms were diarrhea in 12 (16.4%) and vomit in 9 (12.3%) patients. Hiponatremia was present in 39 patients. Mental status alterations were presents in 22 (30.1%) patients. Twenty-one (28.8%) patients were admitted in the intensive care unit. The death rate was 8.2% patients. Chest X-rays showed infiltrate. The predominant pulmonary presentation was unilateral and so in upper as lower lobes. Leucopenia was present in 21 (28.8%) patients. The most frequent treatment established was a fluoroquinolone (levofloxacin) alone or in combination with a macrolide agent. Patients older than 65 years and younger than 65 years did not differ significantly in the clinical presentation and in the outcome. *L. pneumophila* was recovered by culture in 9 (32.1%) patients and only one of them the Binax Now antigen detection was negative because of the serogroup of the isolated was no. 1.

Conclusions: *L. pneumophila* has been increasingly as community-acquired pathogen since our laboratory is performing urinary antigen detection. The disease occurs mostly during the summer to autumn. The death rate was elevated. Levofloxacin is the first line treatment instaurated in our hospital.

P1137 Abstract withdrawn

P1138 Clinical study of acute respiratory infections caused by *Streptococcus milleri* group in the elderly

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Objective: *Streptococcus milleri* group (SMG) is increasingly being recognized as an important pulmonary pathogen. Therefore, we investigated the clinical features of patients with acute respiratory infections associated with SMG.

Methods: Fourteen cases were identified as pulmonary infections caused by SMG. Those clinical and laboratory data from case records were analyzed.

The microbiological diagnosis was based on the results of quantative sputum culture and other invasive procedures, including transthoracic needle aspiration or bronchoscopic examinations.
Results: There were 10 cases of pneumonia, two with both pneumonia and pleuritis, one pulmonary abscess, and one empyema. Nine were men and five were women. Patients' ages were ranging from 65 to 91. The most common symptons at presentation were shortness of breath, cough, sputum and weight loss. An underlying diseases existed in 10 cases. However, four cases had no underlying diseases in the elderly.
Conclusion: We concluded that SMG was more important pulmonary infections than has previously been recognized in the elderly, even if they had no underlying diseases.

P1139 Efficacy and safety of levofloxacin in the treatment of acute sinusitis with high risk of complications (frontal, ethmoid, sphenoid or pansinusitis)

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Objectives: The primary objective was to assess the clinical efficacy of levofloxacin (L) 500 mg once daily, 7–14 days after the end of a 10-day-treatment in acute sinusitis with high risk of complications (SRC) and bacteriologically documented.
Methods: A prospective, international, multicenter, open, non comparative study was conducted from February 2001 to May 2002 by 51 ENT specialists. The intent-to-treat analysis (ITT) has been performed on 174 patients (P) with a diagnosis of SRC confirmed by independent experts on the X-ray/CT scan (frontal: 81%, sphenoid: 9.2%, pansinusitis: 7.5%, ethmoïdo-sphenoid: 2.3%). Pre-therapy swab sampling was systematically performed before treatment under the middle meatus (or at the posterior oropharyngeal wall in case of sphenoid sinusitis). The per protocol analysis (PP) was performed on a subgroup of 101 SRC (58.0%), with a presumed bacterial infection at inclusion (presence of leukocytes/bacteria at the direct microscopic examination of the pus) and without major violations of the protocol.
Results: In the ITT population, the mean age was 39.9 ± 13.2 years [range: 18–74 years] with a sex ratio of 1. One-third of the P had ≥ 1 episode of sinusitis in the year prior to inclusion. In the PP population, cultures were positive in 76 P and among the 102 isolated organisms, the most frequent ones were *S. pneumoniae* (26.5%), Enterobacteriaceae (20.6%), *H. influenzae* (15.7%) and *S. aureus* (13.7%). In this population, clinical success, 7–14 days post-therapy, was observed in 94.1% (95/101) with a 95% confidence interval of [89.5–98.7]. No difference was observed according to the sinus involved: frontal = 77/82 (93.9%), sphenoid = 8/8, pansinusitis = 8/9 (88.9%), ethmoïdo-sphenoid = 2/2. The clinical response at the follow-up visit, 3–5 weeks after the end of therapy, was successful in 85.2% (86/101) and 82.8% (144/174) of the PP and the ITT populations, respectively. The clinical safety was satisfactory in 97% of P: a premature discontinuation of L was observed in six cases, mainly for digestive disorders.
Conclusions: The efficacy and safety of L 500 mg once daily was confirmed in this group of P with acute SRC.

P1140 Epidemiologic studies on the nasopharyngeal carriage of nontypable *Haemophilus influenzae* among healthly children attending day-care centers

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Objectives: Implementation of vaccination against *H. influenzae* type b reduced both morbidity and rates of carriage. It is suspected that in the future it might result in growing importance of carriage and the diseases induced by the NTHI and *H. influenzae* of other capsular types. Thus, studies allowing to recognize relationships in the circulated strains population structure might allow to monitor frequency and turn-over.
Methods: In this study, 112 *H. influenzae* strains from nasopharynx of healthy, not-vaccinated 1–5-year-old-children (and their teachers) attending 11 day-care centers in western part of Poland were isolated during Jan–May 2002. DNA extracted by use of QIAamp Mini Kit (Qiagen) was used for RAPD typing with commercially available RAPD Analysis Beads (Amersham Pharmacia Biotech). DNA extracted by phenol–chlorophorm method was used for AFLP typing with use *Hin*dIII/*Taq*I restriction of the DNA, ligation of restriction products with specific adaptors, and then amplification with primers specific to adaptor/restriction sites with one selective basis. Patterns generated through RAPD and AFLP metods were analyzed with GelCompar Software (Applied Math).
Results: New-designed tool for monitoring of *H. influenzae* genetic diversity by AFLP technique showed higher level of discriminatory power and reproducibility in comparison to RAPD. Intra/intergel reproducibility found for RAPD and AFLP reached 97%/96% and 96%/94% values. The overall genetic similarity definied by the Pearson product-moment correlation coefficient chieved 58 and 35% for RAPD and AFLP, respectively. All encapsulated isolates typed by RAPD were found in a single clade of dendrogram with similarity level of 94%, and in AFLP typing there were found in a single group of nontypeable isolates. In RAPD and AFLP at the similarity level of the 70 and 60%, six clusters were found by both methods used. Similarity level of dendrograms constructed for strains isolated from individual child-care centers achieved higher values in CCC1 (35%) in comparison with CCC6 (74%) in analyzed institutions.
Conclusions: We designed much more sensitive and reproducible system for genetic typing of NTHI strains than found in case of RAPD typing. AFLP tool has potential to monitor turn-over of NTHI strains among individuals.

Infections in transplant patients

P1141 Is *Aspergillus* precipitins testing before lung transplantation of clinical benefit?

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Background: *Aspergillus fumigatus* precipitins are found in a high proportion of patients with pulmonary aspergillosis, especially in patients with aspergilloma or allergic bronchopulmonary aspergillosis. These patients might only grow the fungus sporadically pre transplantation but are at risk of developing severe infection post transplantation. Targeted antifungal prophylaxis pre operatively might be required.
Objective: To establish whether precipitins testing is useful in predicting which patients might develop *Aspergillus* infection post transplantation.
Methods: We surveyed the results of *Aspergillus* precipitins tests performed on 115 patients who underwent lung transplantation between October 1997 and October 2001.
Results: Nine of 115 lung transplant (LTx) recipients were *Aspergillus fumigatus* precipitins positive, 106 patients were negative. Four of the nine precipitins positive recipients grew *Aspergillus* post transplantation from respiratory specimens: all received systemic antifungals when the cultures became positive, but one patient nevertheless died of invasive aspergillosis. All four grew *Aspergillus* from sputum at some time prior to transplantation. Seven of the 106 *Aspergillus fumigatus* precipitins negative recipients grew *Aspergillus* post transplantation: all were treated and no fatality occurred.
Conclusion: Re-isolation of *Aspergillus* from respiratory specimens in the post transplant period appears more likely in AFP positive patients who have grown *Aspergillus* at any time pre lung transplantation. These recipients might benefit from antifungal prophylaxis at the time of transplantation and we therefore recommend *Aspergillus* precipitins testing in these patients.

P1142 Infectious complications in hematopoietic stem cell transplant recipient: experience in a general hospital, Pisa, Italy

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Objectives: To evaluate the incidence, clinical and bacteriologic features of documented infections in Hematopoietic Stem Cell Trasplant (HSCT) recipients.

Methods: The frequency of infectious complications was analyzed in 42 pts with hematologic malignancies who received HSCT from January 2001 to December 2001 at Pisa General Hospital. The median age was 53.3 years (range 27–66). There were 24 males and 18 females. Thirty-three pts underwent autologous HSCT and 9 allogenic HSCT. All pts were received acyclovir, fluconazole and fluoroquinolones as prophylaptic regimen.
Results: A total of 38 infectious episodes were recognized in 22 pts during the early post-HSCT period ($n=27$), and in intermediate or late period post-HSCT ($n=11$). Twelve episodes of febrile neutropenia remained with unknown etiology. As expected, in the late post-HSCT period, the incidence of infectious episodes was higher in allogeneic HSCT recipients than in autologous ones; Infectious complications rate correlated positively with the deepness and length of neutropenia in the early period. There were 21 sepsis (11 coagulase negative staphylococci, 3 *Pseudomonas aeruginosa*, 2 *Serratia marcescens*, 2 *Stenotrophomonas maltophilia*, 2 *Escherichia coli*), 2 pneumonias and 1 vertebral osteomyelitis. All staphylococcus strains were *in vitro* resistant to oxacillin and ciprofloxacin and 8 out of 15 Gram negative rods were resistant to ciprofloxacin. Most of the infectious complications were cured with appropriated antimicrobial therapy and/or with the engraftment and, in 4 cases, with central venous catheter removal. One patient developed a positive CMV antigenemia; a precore mutant form of HBV reactivation was diagnosed in another patient. No cases of invasive fungal infections were recognized. Five pts died, but only one by infective cause (septic shock). Pneumonia was a coexisting cause of death in 2 pts in the late period.
Conclusions: Most of infective complications, occurred in the early period post-HSCT, were due to coagulase negative staphylococci and Gram negative rods resistant to ciprofloxacin. For this reason the uselfuness of fluoroquinolone prophylaxis in HSCT recipients should be re-evaluated.

P1143 Necrotizing fasciitis caused by *Cryptococcus neoformans* in a renal transplant recipient

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Case: A 31-year-old man was admitted to our hospital complaining gradually increasing pain, swelling and erythema in his left leg. He received kidney transplantation from a living related donor six years ago. Post-transplantation immunosuppression consisted of tacrolimus (10 mg/day), mycophenolate mofetil (2 g/day) and prednisone (5 mg/day). On physical examination he had tender, indurated erythematous lesion (25 cm × 8 cm) on the left leg and erythematous induration (3 cm × 2 cm) on anterolateral aspect of right thigh. His laboratory results were as follows; WBC: 15400/mm^3 (95% granulocytes), ESR: 52 mm/h, CRP: 50 mg/dL, creatinine: 2.5 mg/dL. Treatment consisting of penicillin G (6 × 3MU/day) and clindamycin (1800 mg/day) was initiated with a presumed diagnosis of NF. Surgical debridement was carried out to remove necrotic tissue in both legs. Surgery revealed that all soft tissue layers were involved with the exception of underlying muscle. Since the histological examination disclosed neutrophilic infiltration throughout the reticular dermis and subcutaneous adipose tissue with necrosis and PAS-positive budding yeast like organisms, which were concordant with *Cryptococcus*, antibacterial therapy was replaced by liposomal Amphotericin B 5 mg/kg on the 2nd day of hospitalization. On the 5th day of hospitalization culture of tissue revealed *C. neoformans*. Cranium and thorax CTs were unremarkable for cryptococcal infection. Lack of evidence of pulmonary infection or meningitis proposed that the present infection was a localized cryptococcosis. Itraconazole (400 mg/day) was substituted for amphotericin B after 15 days of therapy. On the 17th day of the therapy, the patient underwent skin grafting to involved areas. Itraconozole therapy was stopped at 6th week. At 1-year follow-up, the wounds were completely healed and renal function was normal.
Conclusion: Presence of *C. neoformans* has been described in invasive soft tissue infection very rarely. This case is interesting in many ways. Although it's accepted that cutaneous involvement is a marker of disseminated cryptococcosis, any other infection site and fungemia have not been detected in this case. It has been reported that tacrolimus has a temperature-dependent inhibition on *C. neoformans* that may prevent CNS infection but allow growth of fungus at cooler body sites such as skin. It may explain why this case has only cutaneous involvement. Itraconozole therapy, used in this case, has limited clinical experience in transplant recipients.

P1144 Isolates of 1997–2002 from transplant patients and their antibiotic susceptibilities

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Objective: To evaluate infectious complications and antibiotic use in renal transplant recipients.
Methods: The strains were isolated from the urinary and respiratory tracts of the renal transplant recipients in the transplantation unit at our hospital from 1997 to 2002. The resistance to various antimicrobial agents in a six-year period was searched by disk diffusion according to the NCCLS.
Results: 324 Gram-negative rods, 291 nonfermentative Gram-negative rods, 164 staphylococci and 211 enterococci strains were evaluated in this study. Full susceptibility was observed against vancomycin and teicoplanin in enterococci and staphylococci, and against imipenem and meropenem in Enterobacteriaceae. Trimethoprim-sulfamethoxazole resistance in Enterobacteriaceae; penicillin resistance in staphylococci; piperacillin, gentamicin and netilmicin resistance in nonfermentative Gram-negative rods were higher than the other antimicrobials routinely used in these strains.
Conclusions: The transplant patients are assigned ampiric antibiotics for postop prophylaxis and these antibiotics should be chosen according to the previous susceptibility data.

P1145 Evaluation of diarrhea episodes in renal transplantation patients

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Objective: To review the etiology, clinical features diagnostic procedures and outcome of diarrhea episodes developed in renal transplantation (tx) patients.
Methods and results: Between 2000 and 2002, 42 episodes of diarrhea in 38 renal tx patients (13 female, 25 male) were evaluated for causative agents, diagnostic procedures and outcome retrospectively. The mean age of the patients were 31.8 years, 33 of them received the graft from living donor. The recipient's immunosuppressive protocols included various combinations of prednisolone, cyclosporine, mycophenolate-mofetil, azathioprine and tacrolimus. On initial admission diarrhea was accompanied by fever in 11 episodes and vomiting-nausea in five. Median time for development of diarrhea was 35.5 months after transplantation (3 days−14 years), 35 of the episodes developed late post transplant period (beyond 6 months). Seventy stool samples of 42 episodes were examined for bacterial (*Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Vibrio*, *Clostridium difficile*) and protozoal agents (*Cyriptosporidium*, *Entomoeba*, *Giardia*) by using microscopic examination, cultivation and toxin screening methods. Colonoscopic examination and biopsy was also performed in 16 episodes of diarrhea. The etiology was identified in 22 (52.3%) episodes whereas the other cases remained as self limited with unknown etiology. Among in 22 episodes 12 caused by infectious etiology, 5 immunosuppressive drugs and 5 by other causes (FMF, amyloidosis and colitis) For seven episodes definitive diagnosis were performed by microscopic examination of stool, for two by culture and for six by colonoscopic examination and/or histopathological examination of biopsy specimen. The etiology and diagnostic procedures are shown in Table 1.

Table 1 Etiology and diagnostic procedure of diarrhea episodes

Etiology	Number ($n=42$)	Diagnostic procedure
Infectious	12 (28.6%)	
Giardia lamblia	3	Stool microscopy
Cryptosporidium	4	Stool microscopy (AFB staining)
S. sonnei	1	Culture
Campylobacter jejuni	1	Culture
Entomoeba histolytica	1	Colonoscopy
CMV	1	Coloscopy and Bx
Clostridium difficile	1	Toxin A/B
Non- infectious	10 (23.8%)	
Drug related	5	Exclusion
Others (FMF, amyloid, colitis)	5	Coloscopy and Bx
Undetermined	20 (47.6%)	

In this study we concluded that routine diagnostic procedures have limited values to determine the etiology of diarrhea in renal transplant patients. But colonoscopy may help the diagnosis in these group especially for the etiology other than infectious and drug related causes.

P1146 Detection of *Trichomonas tenax* in renal allograft recipients by PCR-RFLP

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Objectives: Renal allograft recipients (RARs) have problems with their gums and teeth. The aim of the study was to identify parasitic trichomonad in oral cavity of these patients. Because of the insufficiency of conventional techniques (cultivation and microscopic observation) molecular technique (PCR-RFLP) was applied.
Methods: 50 patients (20–70 years old) were investigated: 21 females and 29 males. The material used for PCR-RFLP was saliva and smears taken from the oral cavity. A pair of primers was designed to amplify ITS 1–5.8SrRNA–ITS 2 region and a product of PCR was digested with two restriction enzymes: *Dde*I and *Msp*I.
Results: 12% of patients were positive for oral trichomonad infection: 4 females and 2 males. The PCR product was 393 bp long and after digestion it showed restriction fragments: 136, 125, 50 and 82 bp (*Dde*I cleaving); 198 and 190 bp (*Msp*I cleaving). The size of the product and of the restriction fragments was characteristic for *Trichomonas tenax* – the most frequent trichomonad species occurring in human oral cavity.
Conclusions: The infection of RARs with *Trichomonas tenax* was 12%. Sex-dependent trichomonad infection was noticed: twice as more females were infected than males. Oral trichomonosis in healthy humans is 10–12%. Our results suggest that the infection caused by *T. tenax* in these immunosuppressed recipients is on a similar level as in healthy humans.

P1147 A randomized dose de-escalation study of meropenem in febrile patients with severe neutropenia

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Objective: This prospective randomized study was designed to compare the safety and efficacy of meropenem 0.5 g t.i.d. vs. meropenem 1.0 g t.i.d. after an initial successful treatment with meropenem 1.0 g t.i.d. for 72 h in febrile patients (pts) with severe neutropenia.
Methods: 101 neutropenic patients with different hematological or oncological diseases undergoing allogeneic (90 pts) or autologous (5 pts) hematopoietic stem cell transplantation or chemotherapy (6 pts) received initial meropenem monotherapy (1.0 g t.i.d.) after onset of fever. In case of successful initial treatment (decline of fever, improved signs of infection, bacterial susceptibility to meropenem, respectively) after 72 h without modification of the antibacterial regimen, patients were randomized to receive either a deescalated dose of meropenem (0.5 g t.i.d.) or to continue with the initial dose. Meropenem was administered until the febrile episode resolved or antibiotic treatment was modified according to the clinical condition or results of bacterial diagnostics. A glycopeptide was allowed to be added to the regimen if meropenem-resistant bacteria were detected.
Results: Most patients (86%) presented with fever of unknown origin, and 14% presented with bacteremia, pneumonia or urinary tract infection, respectively. Sixty-six pts (65%) showed an initial response to meropenem monotherapy, and 34 of them were randomized to receive the deescalated dose of meropenem (group 1) for a median time of 9 days (range: 5–23 days), whereas 32 continued with the initial regimen (group 2) for a median of 10 days (range: 5–26 days). The overall clinical response rate at the end of meropenem therapy was 71% in group 1 and 75% in group 2 (P = n.s.). A glycopeptide was administered in 56% (group 1) and 66% (group 2) of randomized pts. Most documented isolates consisted of enterococci and coagulase-negative staphylococci. With regard to safety, no serious adverse effects to meropenem therapy were observed in both groups.
Conclusion: Meropenem monotherapy (1.0 g t.i.d.) is an effective and well tolerated monotherapy in febrile patients with severe neutropenia. Dose deescalation with meropenem 0.5 g t.i.d. after 72 h is considered to be as effective and safe and more cost effective than the recommended standard dose.

P1148 Postoperative infections caused by *Mycoplasma hominis* and *Ureaplasma urealyticum* in patients after renal transplantation

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Objectives: To characterize the infections caused by *U. urealyticum* and *M. hominis* in patients with renal transplants.
Methods: From three patients standard aerobical, anaerobical and mycological cultures were negative despite clinical signs of infection. Wound exudates and abdominal fluid were therefore cultured on A7 medium and *Mycoplasma* IST test (Biomerieux) which detects the presence of mycoplasmas and determines their antimicrobial susceptibility. A7 cultures were incubated in 37°C in microaerophilic bags for 48 h.
Results: Both A7 and *Mycoplasma* IST test were positive in 3 patients. In the first case *M. hominis* was isolated from wound exudate. In patient 2 the abdominal fluid was positive for *M. hominis*. *U. urealyticum* and *M. hominis* were isolated from wound exudate and abdominal fluid obtained from the third patient.
Conclusions: *M. hominis* and *U. urealyticum* are capable of causing serious infections in renal transplant patients. In case of negative standard cultures the presence of mycoplasma should be suspected and appropriate media inoculated.

P1149 De Novo tumor in a pediatric liver recipient associated with Epstein-Barr virus

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Introduction: An increased risk of neoplasia is a well-recognised complication of organ transplantation. Epstein-Barr virus (EBV) infection is associated with significant morbidity in allograft recipients including post-transplant lymphoproliferative disorder (PTLD) but others tumors seems also to be associated with EBV. Leiomyosarcoma is a rare tumor in children with only 4 cases described in pediatric LT recipients. De Novo tumors in LT children are most often drive by EBV infection.
Case report: We describe one case of pediatric transplant recipient who developed neoplasia after transplantation. The first case is a 5-year-old girl who received a liver graft at age of two due to biliary atresia. Two years after transplant developed six hepatic nodules. The histomorphological features were consistent with liver leiomyosarcoma. Blood and biopsy EBV DNA PCR were positives. Reducing immunosuppression and initiating acyclovir therapy was associated with no detection of the EBV DNA in blood by PCR and one year after the liver nodules disappeared.
Conclusion: Pediatric transplant patients have increased risk for EBV-related disease because many are first exposed to EBV after transplantation in the context of potent immunosuppression. EBV serology has a decreased usefulness in the diagnosis. EBV PCR is the most important tool for close monitoring EBV infection and EBV-related tumors.

Urinary tract infections

P1150 Etiologic structure and antibiotic resistance of Gram-negative rods isolated in patients with bacteremia and urinary tract infections in Latvia

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Objectives: To establish etiologic structure and antibiotic resistance of hospital-acquired and community-acquired infections of Gram-negative rods isolated from blood and urine samples.
Methods: Between January 2001 and November 2002 993 Gram-negative rods from blood and urine samples from hospitalized patients in Latvian Infectology Center, Children Hospital 'Gailezers', Hospital of Traumathology and Orthopedics were collected and also urine isolates of community-acquired patients. Strains were identified in BBL SCEPTOR panels and antimicrobial susceptibility were tested by: disk diffusion method (NCCLS), minimal inhibitory concentration (MIC) of an antimicrobial agent in SCEPTOR panels, extented spectrum b-lactamase (ESBL) testing by *E*-test ESBL (BioDisk).
Results: 993 strains were identified as 24 species: 536 *Escherichia coli*, 80 *Klebsiella pneumoniae*, 51 *Klebsiella oxytoca*, 31 *Enterobacter cloacae*, 5 *Enterobacter aerogenes*, 4 *Enterobacter agglomerans*, 30 *Serratia marcescens*, 2 *Serratia liquefaciens*, 32 *Proteus mirabilis*, 18 *Proteus vulgaris*, 10 *Morganella morganii*, 3 *Providencia rettgeri*, 3 *Salmonella enteritidis*, 2 *Yersinia enterocolitica*, 1 *Shigella flexneri*, 86 *Pseudomonas aeruginosa*, 10 *Pseudomonas putida*, 6 *Pseudomonas alcaligenes*, 1 *Pseudomonas cepacia*, 29 *Acinetobacter baumanii*, 21 *Acinetobacter lwoffi*, 1 *Stenotrophmonas maltophila*, 2 *Moraxella osloensis*, 2 *Bordetella bronchiseptica*. The 350 (35.2%) isolated strains were multidrug resistant: 40.2% of hospitalized patients and 14.7% of community patients. In hospitalized patients isolated strains polyresistance was mainly registered of genus *Pseudomonas*—83.0%, *Citrobacter*—58.3%, *Klebsiella–Enterobacter–Serratia* (KES) group—53.1%. In community UTI patients isolated strains multidrug resistance prevailed in genus *Pseudomonas*—85.7% and *Acinetobacter*—42.9%.
Conclusions: The most common pathogen isolates from urine and blood Gram-negative cultures were *E. coli*, genus *Klebsiella* and *Pseudomonas*. Multidrug resistant strains of Gram-negative rods in Hospitals were highest than in community. In hospitalized patients polyresistant strains prevailed in genus *Pseudomonas*, *Citrobacter* and KES group, in community patients—in genus *Pseudomonas* and *Acinetobacter.*

P1151 Resistance to beta-lactam antibiotics and to cephalosporins of strains isolated from urine

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Background: One of the most difficult problems in hospitals is the appearance of resistant bacteria strains to antimicrobial agents. The objective of our study is to describe the antimicrobial susceptibility to frequently used drugs for treatment of urinary tract infections.
Methods: Bacteria were isolated and tested for antibiotic resistance using a panel of 23 beta lactamines and cephalosporins. Antimicrobial susceptibility testing of isolates was determined by disk diffusion method as recommended by NCCLS.
Results: The pathogens identified from urine culture were *Pseudomonas* sp., *E. coli*, *Klebsiella oxytoca*, *Proteus* sp., *Acinetobacter*, *Morganella morganii*. *P. aeruginosa*, fluorescens, *E. coli*, *Enterobacter*, *Acinetobacter* strains showed a high resistance to most of beta lactamic antibiotics but were still susceptible to carbapemens and some broad spectrum penicillin's: ureidopenicillin, ureidopenicillin and beta lactamase inhibitors (*E. coli*, *P. fluorescens*, Enterobacter Acinetobacter), Carbenicillin (*P. aeruginosa*, *P. fluorescens*, *E. coli*), Ticarcillin/CA (*Acinetobacter calloaceticus*), Aztreonam (*E. coli*, Enterobacter). *Proteus mirabilis*, *Klebsiella oxytoca* and *Morganella* strains showed 50% resistances to Ampicilin, Amoxicilin/CA, Klebsiella to Piperacilin and Ticarcillin but all three bacteria were susceptible to all other beta lactamics. *P. aeruginosa* showed a high resistance to Cefpodoxime, Cefixime, Cefazolin, Cephalotin, Cefuroxime, Cefoxitin, Cefotaxime, beeing susceptible only to Carbapenems, Ceftazidime and Cefepime. *P. fluorescens* strains were resistant to Cefpodoxime, Cefixime, Cefotaxime and susceptible to other cephalosporins. *Enterobacter* and *Acinetobacter* were resistant 25–50% to first and second-generation cephalosporins, but susceptible to Cefpodoxime, Ceftriaxone and Cefepime. Klebsiella, Proteus and Morganella did not show resistance to second and third generation cephalosporins.
Conclusions: Only a limited number of beta lactamic antibiotics and cephalosporins show sufficient activity against uro-pathogen Gram-negative bacilli. Resistance to third generation's cephalosporins is very important. Recommended drugs are broad-spectrum penicillins (Carbenicillin), carbapenems (Imipenem Meropenem) and cephalosporins like Ceftazidime and Cefepime. Carbapenems and Cefpirome are effective against all Gram-negative bacilli (*P. aeruginosa* included) producing extended spectrum beta-lactamases and are recommended for therapy of urinary infections.

P1152 A comparative study of the frequency and the antimicrobial susceptibility of causative agents between hospital and community-acquired urinary tract infections

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Objective: To compare the prevalence of the causative agents between hospital and community-acquired urinary tract infections (UTI) and evaluate the differences in antimicrobial susceptibility of the most frequently isolated bacterial strains (*E. coli*, *E. faecalis*).
Methods: Between January 1997 and November 2002, a total of 22.123 urine specimens from hospitalized patients as well as from patients with community-acquired UTI were tested in our laboratory. The cultures were performed by conventional methods. The identification of the organisms to the species level and their susceptibility testing to antimicrobials was performed by the API system (BioMerieux).
Results: A total of 3.785 bacterial stains were isolated. The majority of the isolates came from hospitalized patients. The frequency of the most common isolates in hospitalized patients was: *E. coli* 50%, *P. aeruginosa* 11%, *E. faecalis* 10%, *K. pneumoniae* 8%, *P. mirabililis* 5% and in the community-aquired UTI this was: *E. coli* 68%, *E. faecalis* 8%, *P. mirabilis* 7%, *K. pneumoniae* 6% and *P. aeruginosa* 4%. The in vitro resistance rates of the most frequently isolated bacterial strains (*E. coli*, *E. faecalis*) are shown in the table.

	E. coli		*E. faecalis*	
	H (%)	C-A (%)	H (%)	C-A (%)
Amoxicillin	44	36		
Piperacillin	13	11	4	4
Amox/Clav.	6	3		
Ticarcillim	37	33		
Ticar/Clav.				
Cefulothin	16	10		
Cefoxicin	8	2		
Cefritaxime	3	3		
Ceflazidime	5	3		
Aztreodani	5	3		
Imipenem	0	0		
Gomtamicin	4	3	20	7
Amicacin	2	1		
Nalidixic acid	16	7		
Ciprofloxacin	10	4	51	35
Co-trimoxazole	23	16		
Nitrofurantoin	4	3	9	8
Vanomycin			3	0
Telcoplanin			2	1
Pen Entero (8 mg/L)			7	12
Amp. Entero (8 mg/L)		6	9	
Tetracyclines			64	67
Erythromycin			44	48

In vitro resistance rates
H: Hospital strains
C-A: Community-Acquired strains

Conclusions: The community-acquired *E. coli* strains had lower in vitro resistance rates for all of the antibiotics tested. As for *E. faecalis*, we observed higher resistance rates for Pen. Entero., Amp. Entero., Tetracyclines and

Erythromycin the community-aquired strains, which may show high consumption of these antibiotics in the community.

P1153 Antibiotic resistance in outpatient urinary isolates: interim results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA)

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on behalf of the NAUTICA Group

Objectives: Outpatient urinary tract infections (UTI) are common and are managed empirically. However, antibiotic resistance is increasing to commonly prescribed antibiotics including penicillins, cephalosporins and sulfonamides. The purpose of this study was to assess the prevalence of antibiotic resistance in outpatient urinary isolates from North America.
Methods: Thirty medical centers representing all geographic regions of the United States (US) Bureau of the Census and 10 medical centers from all regions of Canada submitted up to 50 consecutive outpatient urinary isolates to the coordinating reference lab (Health Sciences Centre, Winnipeg, Canada). NCCLS-specified MIC microbroth dilution testing was performed against ampicillin (Amp), trimethoprim/sulfamethoxazole (TMP/SMX), nitrofurantoin (Nitro) and levofloxacin (Levo).
Results: Species prevalence of 1658 of 2000 isolates received to date included: *E. coli* 57.6%, *Klebsiella pneumoniae* 12.7%, *Proteus mirabilis* 5.5%, *Enterococcus* spp. 4.9%, and *Pseudomonas aeruginosa* 2.9%. No difference in bacteriology was reported between the US and Canadian centres. Antibiotic resistance rates (%) among all isolates tested to date ($n = 959$) and *E. coli* only ($n = 552$), respectively, were Amp 41.0 and 36.7%, TMP/SMX 20.5 and 22.3%, Nitro 11.5 and 1.9%, and Levo 8.7 and 6.3%.
Conclusions: Antibiotic resistance in outpatient urinary isolates obtained from medical centres in the US and Canada against all isolates and *E. coli* only, respectively, was Amp 41.0 and 36.7%, TMP/SMX 20.5 and 22.3%, Nitro 11.5 and 1.9%, and Levo 8.7 and 6.3%.

P1154 Incidence and susceptibility of staphylococci in urinary tract infections

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Objectives: To carry out the incidence of coagulase-negative staphylococci (CoNS) and *S. aureus* in urinary tract infections and also to carry out the susceptibility of staphylococci in urinary tract infections.
Methods: During a 2-year period (2001–2002), 312 (8.47%) CoNS strains and 31 (0.84%) *S. aurus* strains were collected from 3684 positive urine cultures. The identification of CoNS strains was carried out by ID 32 Staph and of *S. aureus* by conventional methods. The susceptibility test was performed either by the breakpoint system: mini API or the VITEK system (Biomerieux).
Results: 184 (58.97%) of CoNS strains were isolated from women's urine cultures while 128 (41.02%) CoNS were isolated from men's urine cultures. 167 (53.52%) CoNS strains were obtained from outpatients' cultures while 145 (46.47%) were from inpatients' cultures. 18 *S. aureus* strains were isolated from outpatients' cultures and 13 *S. aureus* strains were isolated from inpatients' cultures while 21 *S. aureus* strains were obtained from women's cultures and 10 were obtained from men's cultures. The susceptibility of CoNS was 100% to vancomycin and teicoplanin, 52.6% to oxacillin, 60% to norfloxacin, 86.5% to nitrofurantoin, 85% to netilmicin, 60.3% to gentamicin, 70.5% to trimethoprim/sulfamethoxazole, 57% to tetracycline, 50% to clindamycin and 33.7% to erythromycin. The susceptibility of *S. aureus* was 100% to vancomycin and teicoplanin (28/31) to nitrofurantoin (26/31) to trimethoprim/sulfamethoxazole (23/31) to oxacillin, norfloxacin and gentamicin (25/31) to netilmicin, (22/31) to clindamycin (20/31) to tetracycline and (19/31) to erythromycin.
Conclusions: CoNS are often responsible (8.47%) for urinary tract infections in women, specially in outpatients. *S. aureus* is rarely responsible (0.84%) for a urinary tract infection. Susceptibility to vancomycin and teicoplanin was absolute for CoNS and *S. aureus* and variable to the other antimicrobial agents.

P1155 *E. coli* recurrent urinary tract infection in children: relapse or reinfection?

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Background: In childhood urinary tract infection (UTI) is one of the most frequent bacterial infections and almost half of the patients have a recurrence following the initial infection. The recurrences are either relapses or reinfections and in most cases due to *E. coli*. The objective of this study was to assess the potential for differentiating episodes of relapse and reinfection in children with recurrent urinary tract infection (RUTI) during an 1-year follow-up by applying pheno- and genotyping to compare index and consecutive *E. coli* strains. Furthermore, we attempted to estimate whether clinically symptomatic UTI episodes and patients with vesicoureteric reflux (VUR) are more prone to relapse than reinfection.
Materials and methods: Altogether 77 *E. coli* strains isolated from 26 children with RUTI were examined in this study. We applied antibiotic susceptibility tests utilising six commonly used antibiotics for paediatric UTI treatment in order to phenotype strains. Using the antibiotic susceptibility pattern we could not detect cases of relapses. To detect genotypic similarity a random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) was used with two different primers applied.
Results: We found that the persistence of genotypically defined *E. coli* strains occur in only one-quarter of RUTI episodes in children. The clinical manifestation of RUTI and the presence of VUR could not predict the persistence of a defined genotype of *E. coli*.
Conclusion: Genotyping of consecutive *E. coli* strains enables to differentiate cases of relapse and reinfection, which may be important in choosing an appropriate intervention strategy for children suffering from RUTI.

P1156 Urinary tract infection in community-based patients. Most frequent bacterial pathogens and susceptibility patterns. Results from a Brazilian study

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Objectives: The goal of this study was to assess the most frequent pathogens responsible of urinary tract infections (UTI) in community patients and the pattern of antimicrobial resistance in Sao Paulo, Brazil.
Methods: A 2-year period (January 2000 to December 2001), 142 502 urine cultures from community-based patients were analyzed. All positive cultures that presented one single pathogen with counts of equal or above 100 000 CFU/mL were considered for the analysis. Positive cultures from the same patient were only taken into account if the cultures were performed at least 30 days apart. Based on these criteria, 18 298 (12.8%) positive cultures were analyzed in this survey of which 88.3% belonged to females and 11.7% were from male patients.
Results: Among the 18 298 positive cultures analyzed, 88.5% presented growth of Enterobacteriaceae followed by 7.7% of Gram-positive cocci. Among Enterobacteriaceae, *E. coli* (72%) was the most frequently isolated followed by *K. pneumoniae* (6.1%) and *P. mirabilis* (6.0%). Among Gram-positives, *E. faecalis* was the most frequent (4.7%). In males it was detected a higher incidence of UTI in infants (26.1% below 4 years old) and in the elderly (19.3% above 60 years old). By analyzing the susceptibility profile, we observed an important resistance in *E. coli* towards ampicillin (43.1%) and cotrimoxazole (33.5%). It was also noticed a significant variation of resistance to cotrimoxazole between ages (29.5–53.0%). Concerning norfloxacin, it was observed a higher resistance in males (19.9%) than in females (9.4%). In *E. faecalis* isolates, 14.9% were found to be resistant to ciprofloxacin, 62.1% to tetracycline, and 1.1% to nitrofurantoin. A higher resistance to all antimicrobials tested against *E. faecalis* strains was observed in patients above 60 years old.
Conclusions: Apart from analyzing the most frequent UTI pathogens, differences in resistance profile among isolates of different age intervals and genders were also noticed, reinforcing the hypothesis that empiric treatment of patients with symptoms of UTI may contribute in the selection of resistant strains.

P1157 Uropathogens and their antibiotic resistance in outpatients in a general hospital, Greece

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Objectives: To study the etiology of urinary tract infections (UTI) and the antibiotic resistance of uropathogens isolated from outpatients.
Methods: During 1-year period (1/11/01–1/11/02), 2150 urine samples were obtained from outpatients with clinical symptoms of acute cystitis in a tertiary General Hospital of Athens. Urine cultures were performed according to standard techniques. Urinary isolates were identified using API and Crystal ID systems, while the susceptibility of them was tested by Kirby-Bauer method.
Results: 714 out of 2150 urine cultures (33%) fulfilled the criteria for significant bacteriuria. (Pure growth of >100000 cfu/mL urine for bacteria and >1000 cfu/mL for *Candida* sp.) There were four groups of causatives agents: (1) Gram-negative rods: the family Enterobacteriaceae 590 (82.6%), from them *E. coli* 464 (64.8%), *Klebsiella* sp. 44 (6.1%), *Enterobacter* sp. 8 (1.1%), *Serratia* sp. 2 (0.28%), *Proteus mirabilis* 72 (10%). (2) Gram-positive cocci 76 (10.6%), from them *E. faecalis* 30 (4.2%), *E. faecium* 2 (0.2%), *St. epidermidis* 28 (3.9%), *St. saprophyticus* 4 (0.5%), *St. aureus* 12 (1.6%). (3) Gram-negative nonfermenters 36 (5%), from them: *Ps. aeruginosa* 32 (4.4%), *Acin. baummanii* 4 (0.5%). (4) *Candida* sp. 12 (1%). The resistance of isolated strains to antibiotics was as following: *E. coli* and *Klebsiella* sp. were resistant to ampicillin (24.1–100%), amoxicillin-clavulanicacid (3.4–15.9%), cephalothin (6–18.1%), nitrofurantoin (1.7–22.2%), cotrimoxazole (8.6–22.7%) quinolones (1.7–13.6%) retrospectively. The resistance of *Ps. aeruginosa* to imipenem was 12.5%, ceftazidime 18.7%, meropenem 9%, quinolones 46% and aminoglycosides 34%. The resistance of coagulase-negative Staphylococci (CoNS), to oxacillin was 37.5%, gentamycin 6.2%, cotrimoxazole 29%, quinolones 21.8%. *Enterococcus* sp. was resistant to ampicillin 6.2%, gentamycin H-L 12.5%, quinolones 21.8%. There was no resistance to glycopeptides for CoNS, as well as *Enterococcus* sp.
Conclusion: *E. coli*, *Proteus mirabilis*, *Klebsiella* sp. and *E. faecalis* were the most common uropathogens isolated from outpatient samples. About 24% of isolates of *E. coli* were resistant to ampicillin while the resistance to quinolones, furans, cotrimoxazole was ranged between 1.7 and 8.6%.

P1158 Epidemiology of community-acquired urinary tract infections in the Zenica-Doboj Canton, Bosnia and Herzegovina

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Objectives: The aim of this study was to investigate the epidemiology of antimicrobial resistance of community-acquired urinary tract infections (UTIs) in the Zenica-Doboj Canton, regarding the age, gender, microorganisms identified, repeating of isolates and some other factors, and to propose empiric therapy.
Methods: It was analyzed for 54.638 consecutive urine samples by standard procedures in 1998–2001 period. Antimicrobial susceptibility was performed by disc-diffusion method to 11 antimicrobials.
Results: A total number of 11.012 *E. coli* and other coliforms were isolated, of which 5149 (46.8%) were potentially repeated ones. Male/Female incidences were 1.0 and 4.3/1000/year, respectively; the highest were in the youngest age group, 7.7/1000/year. The most isolates obtained from patients under 20 years, 74.7% in male and 66.1% in female. The differences in susceptibility rates calculated including or excluding repeat isolates were about 1.0%. Inclusion of coliforms other than *E. coli* (25.6%) have increased baseline estimates of resistance in all tested antibiotics except ampicillin and cotrimoxasole (Sxt), for which it was almost equally high in both group tested (70.0 and 50.0%). *E. coli* resistance rate was markedly higher in males than in females, except in the case of ampicillin and Sxt, for which, again, equally highest in both groups. Resistance rate patterns according the age were similar for male/female and *E. coli*/other coliform subsets, lowest for fluoroquinolones, and highest in 20–64 and under 65 age groups.
Conclusions: The high ampicillin (74%) and Sxt (49.3%) resistance rates for all subsets analyzed (*E. coli*/other coliforms, male/female) brings fluoroquinolones on the top of empiric therapy list. Empiric therapy with Sxt in our region seems inadequate even in low-risk population category, and should be left. Nitrofurantoin also should be considered as the first-line therapy especially in children. The ease of procuring antibiotics in this region after the war without a prescription could result in uncontrolled self-medication. It can explain decreasing trend in ampicillin- and Sxt- resistance rates and increasing in fluoroquinolones in this, after the war period. It is important for physicians to know susceptibility data of UTIs and when empiric treatment should start.

Chlamydia and other sexually transmitted infections

P1159 The effects of lipopolysaccharide on human spermatozoa

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Objectives: The spermicidal activity of bacterial endotoxin (LPS) has been reported by different researchers. LPS essentially consists of lipid A, a core polysaccharide (Kdo) and O antigen. The expression of the LPS coreceptor CD14 was previously shown on spermatozoa to be a functional component. Previous work in our group showed that chlamydial LPS revealed a more potent spermicidal effect than that from *E. coli*. That chlamydial LPS was spermicidal was demonstrated by the use of polymyxin B to inhibit its effects. As such, we have extended our initial studies to expand our understanding of the interaction between LPS and human spermatozoa in vitro.
Methods: Semen samples that were identified to be normozoospermic by WHO criteria were obtained from donors attending the Andrology Laboratory, Jessop Wing, Royal Hallamshire Hospital. From each sample, a highly motile suspension of spermatozoa was obtained. Extraction of LPS from *C. trachomatis* serovars E and LGV was performed, other LPS and constituents were obtained commercially. Spermatozoa were adjusted to 5×10^6 million/mL and incubated for 6 h at 37°C/5% CO_2 with 0.1 μg/mL of chlamydial LPS or 50 μg/mL of *E. coli* LPS, lipid A or Kdo. The effects of antihuman CD14 antibody on the combinations of LPS/sperm were investigated. Moreover, the mechanism of death in sperm was studied by flow cytometry.
Results: The toxic effects of *E. coli* LPS were blocked by antihuman CD14 antibody. Percent values of killing for sperm incubated with LPS alone or sperm treated with anti-CD14 antibody for 30 min and incubated with LPS, were 27 and 12%, respectively. When sperm were incubated with 50 μg/mL of synthetic Kdo, some spermicidal activity was shown. Sperm death at 6 h when compared with the control was 20 and 10%, respectively. Lipid A was also shown to have a similar spermicidal activity. The level of apoptosis in the control sperm was 5%. This value changed to 27 and 22% when sperm were incubated with the LPS extracted from *C. trachomatis* serovars E and LGV, respectively.
Conclusions: In conclusion, these data provide a further understanding of the mechanisms of death in human spermatozoa when induced by LPS. There were comparable effects on sperm of both lipid A and Kdo. The spermicidal activity of LPS from *E. coli* was blocked by anti-CD14. Flow cytometry showed that LPS was responsible for the induction of apoptosis in spermatozoa and that this was a prime cause of death.

P1160 Potential adhesins of *Chlamydia trachomatis*

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Objectives: A number of adherence mechanisms in *C. trachomatis* were examined, including outer membrane proteins, lipopolysaccharide (LPS) and heparan sulfate (HS). Our aim was to shed further light on possible *C. trachomatis*–host interactions.
Methods: Two *C. trachomatis* serovars LGV1 and E64 were examined using two cell lines, HeLa and Hec1B. A number of approaches were used which included inhibition of sulfation by growing the cells in the presence of various concentrations of sodium chlorate. To investigate whether HS was present on the chlamydia or not, the effect of chlorate on the EBs was examined. For the reversal of the sulfation inhibition, chlorate treated cells were supplemented with sodium sulfate. To explore the surface proteins, chlamydiae were assayed

for infectivity after treatment with various concentrations of trypsin. A polyclonal anti-MOMP was used to examine the possible role of MOMP. Finally, we used polymyxin B (PmB) as an inhibitor of LPS.
Results: For the LGV1 serovar, infectivity decreased with increasing chlorate concentration. Using 70 mM chlorate, minimum infectivity was obtained in both cell lines. Infectivity of E64 was essentially unaffected in the presence of chlorate. Growing chlamydia in the presence of up to 70 mM chlorate did not seem to have an inhibitory effect on the infectivity of any of the tested serovars. Supplementing sodium chlorate-treated cells with sodium sulfate led to partial restoration of the infectivity. Serovar LGV was sensitive to trypsin even at very low concentrations; whilst serovar E was less sensitive. Use of anti-MOMP antibody resulted in a greater decrease in infectivity of LGV1 than E64. Growing chlamydia in the presence of 100 μg/mL PmB led to a significant decrease in infectivity of both serovars.
Conclusions: Chlorate inhibition of infectivity on the cell lines rather than on chlamydia itself suggested that HS is present on the host cells rather than *C. trachomatis*. Serovar LGV1 was also affected by trypsin treatment more than serovar E. Interestingly, the same pattern of results was seen when an anti-MOMP antibody was tested. This suggests that MOMP may play a role in infectivity. Finally, polymyxin B also showed a marked reduction in infectivity of both serovars, suggesting that LPS might be important. Our findings suggest that serovar LGV rather than E is perhaps more dependant on the infectivity factors we investigated. Moreover, several factors may be implicated.

P1161 Clinical evaluation of VIDAS PROBE *Chlamydia trachomatis* test from endocervical swab specimens in screening high and low prevalence populations

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Objectives: Genital *C. trachomatis* infection, a substantial public health problem, is the most common sexually transmitted disease (STD) in many countries and may cause serious reproductive and ocular complications. Due to the often asymptomatic nature of chlamydial infections, it is important to target screening that includes rapid and accurate diagnosis in order to decrease disease prevalence. This study assessed the clinical performance of the VIDAS PROBE *C. trachomatis* (CT) test as compared with Gen-Probe AMPLIFIED *Chlamydia trachomatis* Assay (AMP-CT) and the BD ProbeTec (PT) CT assay for detection of chlamydial infections from endocervical swab specimens.
Methods: Two patient populations were screened. 'Wishard 1' consisted of patient who presented at a 24H emergent OB-GYN facility that doubles as an STD screening clinic. 'Wishard 2' consisted of patients who presented to their primary care clinician for routine annual or prenatal visits. Three separate randomized endocervical swabs were collected from study subjects (>13 years of age) and transported to the laboratory where each assay was performed according to manufacturer's recommendations. Patient infectivity status was based upon endocervical swab results from PT and AMP-T.
Results: A total of 310 subjects were screened at 'Wishard 1' with 44 classified as asymptomatic and 266 symptomatic. The prevalence rate in the asymptomatic population was 15.91% while the prevalence in the symptomatic population was 8.65% and the overall prevalence was 9.7% (30/310). The VIDAS PROBE exhibited 100% sensitivity (30/30) and 100% sensitivity (278/278) in both asymptomatic and symptomatic subjects. The negative and positive predictive values were also 100%. A total of 297 subjects were screened at 'Wishard 2' with 125 classified as asymptomatic and 172 symptomatic. The prevalence rate in the asymptomatic population was 2.4% while the prevalence rate for the symptomatic population was 5.23%. The VIDAS PROBE exhibited 100% (12/12) sensitivity and 100% specificity (284/284) in both asymptomatic and symptomatic subjects. The negative and positive predictive values were also 100%.
Conclusions: Results from this study suggest the VIDAS PROBE CT is a sensitive and specific test for the detection of CT infections and can be used as a screening test in both high and low prevalence patient populations.

This product has not been cleared by the US FDA and is not yet available for commercial use.

P1162 Detection of *Chlamydia trachomatis* and *Neisseria gonorrheae* from CyTyc PreservCyt ThinPrep Pap smear collection kits using the Gen-Probe APTIMA Combo 2 Assay

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Objectives: To determine the feasibility of utilizing a single collection device that can be used for Pap smear preparations and to screen for sexually transmitted diseases. The CyTyc PreservCyt ThinPrep (TP) liquid Pap smear media is used to collect and transport cervical tissue used in Pap smear preparation and testing for cervical cancer. The cervix is also a site of infection for *Chlamydia trachomatis* (CT) and *Neisseria gonorrheae* (GC) in women. If undiagnosed or untreated, women may acquire cervicitis, urethritis, salpingitis, proctitis, and endometriosis leading to pelvic inflammatory disease (PID), which is a contributor to infertility. These organisms can be transmitted in the birth canal, potentially resulting in conjunctivitis and/or pneumonia in newborns.
Methods: This study evaluated the performance of APTIMA Combo 2 Assay (AC2)(Gen-Probe, Inc., San Diego, CA) for detection of CT and GC from aliquots of the TP solution as compared with detection of these pathogens from routine cervical swab and urine specimens. AC2 is an amplified nucleic acid test that utilizes target capture specimen processing followed by transcription-mediated amplification and dual kinetic assay detection. 159 female subjects have been tested to date. Prior to examination and collection of cervical specimens, subjects collected 10–30 mL of urine. Clinicians obtained subsequent TP and cervical swab specimens during the pelvic examination. The AC2 assay was performed on all swab and urine specimens according to manufacturer's recommendations. Additionally, the AC2 assay was performed on a 1-mL aliquot from the TP solution. Discordant specimens, all positive and an equal number of negative specimens were also tested using the COBAS Amplicor assay (CA) for CT and GC (Roche Diagnostics, Indianapolis, IN).
Results: In this low prevalence population, 11 patients were positive for CT and 1 for GC in both cervical and urine AC2 testing. The corresponding TP aliquot showed complete agreement (100% sensitivity) with no false positive TP results for the remaining patients with AC2 negative swab and urine specimens (100% specificity). The CA assay also showed complete agreement (there were no discordant specimens to test).
Conclusions: Preliminary results from this study indicate the CyTyc PreservCyt ThinPrep medium is compatible with the APTIMA Combo 2 assay, making possible the pairing of highly accurate CT and GC detection with Pap smear testing from a single sample.

P1163 *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* infections in patients with nongonococcal urethritis

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Objectives: *Chlamydia trachomatis* and genital mycoplasmas are known pathogens causing nongonococcal urethritis (NGU). Our objective was to study the distribution of infections with *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* in male with NGU and their respective female partners The study enrolled 926 male patients and 512 female between January 1999 and December 2001 according to the age group.
Methods: Amplicor polymerase chain reaction and direct immunofluorescence assay were used for detection *C. trachomatis* on urethral or/and cervical swab specimens. Presence of genital mycoplasmas in patients' specimens were diagnosed by culture screening.
Results: The overall prevalence of *C. trachomatis* was found in 18.5% (266) of cases of urethral discharge: in 21.0% (195/926) of male with NGU and in 13.8% (71/512) of female partners, respectively. The highest prevalence was determined in the age group of 15–30 years ($P < 0.05$). For urogenital mycoplasma infections it was screened 891 male with NGU and 377 female. Among them genital mycoplasmas were present in 290 (22.8%) of patients, namely in 17.3% of men and in 39.8% of female. *Ureaplasma urealyticum* was

detected in 22.9% of patients with NGU while *M. hominis* was found in only 1.1%. Multiple infections with genital mycoplasma and *C. trachomatis* were also observed in 4.9% of patients.
Conclusion: *C. trachomatis* and *U. urealyticum* infections are common among patients with NGU in our population. *M. hominis* was identified very rarely.

P1164 Incidence of infection by *Chlamydia trachomatis* in female commercial sex workers in Kampala, Uganda

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Objectives: To determine the incidence of cervical varginal infections caused by *C. trachomatis* (CT) in female commercial sex workers (FCSWs), associated factors and practices as well as other sexual transmitted infections.
Methods: Blood and cervicovagina samples were taken from 750 FCSWs attending an STD/STI detection clinic in Kampala city from January 2001 to July 2002. Structured questionnaires with social demographic data, history STI, sexual practices and condom use was applied. Genital exam in all cases and the following STIs detection:condylomatosis (clinical), *Chlamydia trachomatis* infection (direct immunoflurescence), gonorrhea (thayer-martin culture), bacterial vaginosis, trichomoniasis and candidiasis (smear/KOH/Gram).
Results: 750 FCSWs were studied. Mean age 21 years old (range 19–45 years), age starting sexual activity 19, in completely elementary schools 65%, average clients per a week 60 and unstable couples. 54.2% (164) reported condom use with their clients, 18% (57) with stable couples and 17% (51) reported condom use in the French way (same condom for oral and genital sex with more than one woman) Endocervical sample were positive for CT in 23% (207) from which 31% (97) reported no clinical manifestation, 15.8% have mucopurulent cervicitis. 51% reported previous STIs, more frequently bacterial vaginosis and trichomoniasis.
Conclusion: CT infections among FCSWs is high, event with no clinical manifestation. This can increase the risk of HIV transmission as well as other STIs: regardless the cost of this test, incorporation of this type of studies for risky practices, population is very important in order to give early treatment to avoid complications.

P1165 Study of urethral *Chlamydia* infection in men with nongonococcal urethritis in Tehran, Iran

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Objectives: The objective of this study was to determine the rate of Non-gonococcal Urethritis (NGU) caused by *Chlamydia trachomatis* in males referred to the Tehran University of Medical Sciences affiliated hospitals.
Methods: The present study is designed to define the incidence rate of urethral infections with NGU caused by *Chlamydia trachomatis* in patients referred to Tehran University of Medical Sciences affiliated hospitals during April 1999 to August 2000. In this period 310 males aged 20–50 years old, with NGU were included in this study. The patients were referred to the laboratory by the physicians for further microbiological examination. The enrolled patients were interviewed by a physician, and if they agreed to participate in the study an endo-urethral swab and a blood sample were taken from each patient. Blood samples from 200 healthy blood donors were used as control. The endo-urethral swab was transported into a 2SP media and shaken well. Then the 2SP medium containing the swabs were transferred to the School of Public Health Chlamydia laboroatory. The media was then cultured in two confluent McCoy cell monolayers treated with cyclohexamide. After 3 days one of the McCoy cell monolayer was stained by Giemsa technique. If there was no growth of *Chlamydia* then the next passaged for two times. Blood samples were used to check the presence of anti-*Chlamydia* antibodies (IgM and IgG) by indirect immunoflurecent technique (micro-IF).
Results: The results indicated that *Chlamydia* was isolated from 36 out of 310 (11.6%) of patients. The IgM specific anti *Chlamydia* antibodies were detected in a titer of 1/8 in 10 patients (3.2%). The IgG specific anti *Chlamydia* antibodies were detected in a titer of 1/32 and above in 68 patients (21.9%). No IgM anti *Chlamydia* antibody was detected in any of the sample taken from the control group. The IgG anti *Chlamydia* antibodies were detected in a titer of 1/32 and above in 4 (2.0%) of the control group.
Conclusions: All the patients except one with a positive culture resultsshowed a positive titer of IgG. Three of the patients with positive titer of IgM showed a positive culture. were positive. In Western countries most of the NGU cases are caused by *Chlamydia trachomatis* but in this study based on the culture results a smaller portion of NGU cases are caused by *Chlamydia trachomatis* in Iranian society.

P1166 Detection of *Chlamydia trachomatis* DNA and human papillomavirus DNA in cervical samples with regard to vaginal colonization with lactobacilli

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Objectives: The studies aimed at evaluating the risk of *Chlamydia trachomatis* and human papillomavirus (HPV) infections in patients with vaginal flora.
Methods: The studies were performed on 97 women, aged 25–43 years. Vaginal flora was examined by Gram's stain score on basis of standardized criteria (Nugent's score). A score equal and more than 4 was used to to define abnormal vaginal flora. In addition, presence of lactobacilli was confirmed by culture. *Chlamydia trachomatis* DNA was detected in cervical samples using PCR amplification assay (amplification of DNA fragment which encoded omp-1 gene). HPV DNA oncogenic types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58 were detected in cervical samples using PCR-ELISA assay (Sharp Signal System, Digene).
Results: On the basis of vaginal flora evaluation, to subgroups of the women were distinguished. Subgroup 1 included 37 patients with normal vaginal flora characterized by domination of vaginal lactobacilli (score <4). Subgroup 2 included 60 patients with abnormal vaginal flora characterized by the presence of individual lactobacilli or by their absence (score equal and more than 4). In the subgroup 1, *Chlamydia trachomatis* DNA was detected in 9 patients, HPV DNA in 2 patients while in the subgroup 2 *Chlamydia trachomatis* DNA was seen in 3 patients, HPV DNA in 13 patients.
Conclusion: The obtained results may indicate that vaginal colonisation with lactobacilli does not protect the patients from *Chlamydia trachomatis* infection, but reduce the risk of HPV infection.

P1167 Role of *Chlamydia trachomatis* and *Mycoplasma* spp. in rheumatologic disorders and related syndromes

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Objectives: To identify the potential etiologic role of *Chlamydia trachomatis* and *Mycoplasma* in precipitating rheumatological-like disorders.
Methods: At the Clinic of Infectious Diseases of Chieti University 119 patients (82 F and 37 M; mean age: 37.5 ± 10 year) complaining of localized or diffuse arthralgias associated to fever and/or myalgias and/or prolonged fatigue were examined. All subjects underwent routine laboratory and rheumatologic investigations, determination of antibodies towards hepatitis B and C viruses, HIV, *C. trachomatis* and *Mycoplasma* spp., and an urethral swab to search for *C. trachomatis*, *U. urealyticum* and *M. hominis*.
Results: The diagnosis formulated for the 119 patients was: chronic fatigue syndrome (CDC, 1994) in 33 cases (27.7%), fever of unknowned origin in 44 cases (37.0%), idiopathic chronic fatigue (CDC, 1994)in 7 cases (5.9%), HCV or HIV infection in 12 cases (10.1%), reactive arthritis in 13 cases (10.9%) and fibromyalgia syndrome in 10 cases (8.4%). In 64 of the 119 patients (53.8%) an urogenital infection was identified and due to *C. trachomatis* in 19 cases (29.7%), *M. hominis* in 5 cases (7.8%) and *U. urealyticum* in 40 cases (62.5%). All 64 patients underwent an antibiotic therapy (doxycycline or azythromicin). The urethral swab repeated after such therapy showed an eradication of the infectious agent in most of the patients (85.9%). In 9 patients (14.1%), however, a complete resolution of the infection was reached after a second cycle of antibiotic therapy (azythromicin or chlarythromicin in only one case). Furthermore, 23 of the 64 patients (35.9%) showed a clinical benefit from the treatment with a complete remission of the symptomatology they complained of.
Conclusion: Our findings suggest that the determination of sexually transmitted infectious agents must be included in the diagnostic protocol of patients complaining of rheumatologic-like disorders. In fact *Chlamydia trachomatis* and *Mycoplasma* spp. often induce extragenital manifestations in the absence of a local symptomatology. So it is important to identify and treat such silent sexually transmitted infections to prevent potential severe complications (e.g. PID, infertility, etc.) as well as to reach a better understanding of pathophysiologic mechanisms involved in the onset of rheumatologic disorders and related syndromes (e.g. chronic fatigue syndrome, etc.).

P1168 *Chlamydia trachomatis* in rheumatologic patients

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Objectives: Reactive arthritis (RA) triggered by sexually trasmitted infections is referred to as sexually acquired reactive arthritis (SARA). There is no gold standard for the diagnosis of SARA. None of the tests or the clinical symptoms alone are strong enough to make a definite diagnosis of SARA. Tests to identify *Chlamydia trachomatis* together with typical clinical symptoms may be helpful in making the final diagnosis of RA.
Methods: Our study comprised 87 patients, aged 19–78 (58 women, 29 men), hospitalized in one of the rheumathological ward in Wroclaw from 1 May 2000–30 April 2002. The control group consisted of 30 individuals, aged 27–70, without any arthritis complaints. Altogether 117 specimens from urethra were tested by direct immunofluorescence – DIF (IF test, Medac, Germany) as well as 117 serum samples were examined by immunoenzymatic method – ELISA (Viro-Immun Labor – Diagnostika, GmbH) for specific anti-*Chlamydia trachomatis* IgG and IgA antibodies.
Results: Positive DIF results were found in 42 (48%) of the 87 patients, while specific IgG in 56 (64%) and IgA in 16 (18%) of them. Bacteriological and serological findings together with clinical data allowed to diagnose 38 (43.7%) patients as having RA, 5 (5.7%) cases as suspected of having RA (according to Fendler's criteria) and in 44 cases to exclude RA. In the control group ($n = 30$) there were 3 (10%) DIF (+), 8 (26.6%) IgG (+) and 4 (13.3%) IgA (+). Correlation of positive results of 3 tests (DIF, IgG and IgA) was observed in 7, and of negative results in 22 patients. Correlation of positive results of 2 tests (DIF and IgG) was noticed in 29 out of 87 cases. Laboratory positive tests for *Chlamydia trachomatis* allowed to change initial diagnosis in 23 patients from rheumatoid arthritis ($n = 9$), osteoarthritis ($n = 5$), connective tissue disorders ($n = 4$), gout ($n = 1$) and ankylosing spondylitis ($n = 4$) for RA as a final diagnosis. Basing on negative results in 2 cases there was a change from reactive arthritis to reumatoid arthritis.
Conclusions: (i) Positive results of testing for *Chlamydia trachomatis* allowed to diagnose RA in nearly a half (49.4%) of tested rheumathological patients. (ii) High percentage of positive *Chlamydia trachomatis* results in the control group may be connected with a relatively high frequency of chlamydial infections in Lower Silesia Region of Poland.

P1169 Microbiologic characteristics of 25 *Neisseria gonorrheae* strains isolated in a 10-year period

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Objectives: To describe microbiological characteristics of *Neisseria gonorrheae* isolated between 1991 and 2002. Half of the cases belongs to the calendar year 2002; differences between these strains and those of preceding years were compared.
Methods: We included 25 *N. gonorrheae* isolates obtained during the period January 1991–December 2002. We studied beta lactamase production, susceptibility (broth dilution) to penicillin, cefoxitin, ceftriaxone, ciprofloxacin, tetracycline and spectinomycin, and serogroup by monoclonal antibodies technique.
Results: A total of 25 *N. gonorrheae* strains were isolated. In the period 1991–2001, 13 strains (10 from men urethral samples and 3 from women endocervical samples); in 2002 11 strains were isolated from men (urethral samples) and 1 from a woman (endocervical). The mean age was 33 (1991–2001) and 35 (2002), respectively. The serogroup distribution was IA 5 cases, IB 8 cases (1991–2001) and IA 2 cases, IB 8 cases (2002). Beta lactamase production was found in 4 cases in the first period and in one strain in 2002. In 1991–2001 penicillin MIC were >1 in 5 cases, <0.06 in 2, and the rest of strains between these values; in 2002 all the strains had MIC values between 0.06 and 2. In the first period, ciprofloxacin was tested only in three cases: one was resistant and two susceptible; in 2002, all the strains were susceptible (MIC < 0.06). 7 strains were tetracycline resistant (MIC > 1) in the first period, and in 2002 2 of 10 strains tested were resistant. All the strains in both periods were susceptible to cefoxitin, ceftriaxone and spectinomycin.
Conclusions: We detected a progressive rise in the number of *N. gonorrheae* isolates, especially in the last year: we found no differences between the strain characteristics studied in both periods. Beta lactamase production is not very important, but penicillin susceptibility is decrease, probably by other resistance mechanisms. The other classic antibiotic, tetracycline, is also not very effective in vitro. The MIC for the CDC recommended antibiotics (cephalosporins and quinolones) are still below the resistance cut-off.

P1170 *Spontaneous peritonitis* caused by *Neisseria gonorrheae*: a case report

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Gonorrhea is one of the oldest human sexually transmitted diseases. The incidence of the infection is now declining rapidly in industrialized countries. Up to 90% of women with gororrhea may be asyptomatic although sometimes endocervicitis, vulvovaginitis, urethritis, inflammation of Bartholin's glands and even rarely disseminated gonococcal peritonitis may appear. We describe a case of *Spontaneous peritonitis* in a sexually active woman caused by *Nesseria gonorrheae*. A 23-year-old woman was admitted to the emergency department with clinical signs of acute abdomen and increased body temperature, started 2 days before. No infectious focus was reported. During physical examination fever, tachycardia and rebound tenderness of the abdominal wall were present while bowel sounds were absent. On admission, white blood cell count was increased with a shift to the left, CRP was elevated, while the abdominal X-ray was compatible with peritonitis. The patient was taken to the operating theatre for exploratory laparotomy where lavage of the purulent exudate with saline solution and removal of both fallopian tubes took place. Purulent exudate cultures obtained intraoperatively, revealed heavy growth of *N. gonorrheae* while repeated blood cultures were negative. She was treated with metronidazole 500 mg IV, every 8 h, in combination with ceftriaxone IV, 1 g every 12 h. The patient was discharged after 15 days of hospitalization. In conclusion, *Spontaneous peritonitis* is a rare complication of gonococcal infection that provides highly suggestive evidence of transfallopian route of transmission, mainly in previously asymptomatic patients. It is important therefore to consider this route of infection in a sexually active woman with clinical and laboratory features of bacterial peritonitis and include culture media appropriate for *N. gonorrheae* before initiating empirical antibiotic therapy.

P1171 Case finding strategies in a syphilis outbreak

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Introduction: Between January 2000 and September 2002, 526 cases of syphilis have been notified to the National Disease Surveillance Centre through enhanced surveillance of syphilis in Ireland. These cases occurred predominantly in MSM (79%) and were diagnosed principally in the sexually transmitted infection (STI) clinics in the Eastern Regional Health Authority (ERHA)(89%).
Methods: The Director of Public Health in the ERHA established an outbreak control team in October 2000. The number and duration of STI clinics were increased and an additional health advisor was appointed for contact tracing. Community involvement through specialized outreach work, on-site serological testing for syphilis and education was implemented.
Results: STI services were extended in St. James's Hospital and in the Gay Men's Health Project (community clinic). Between 2000 and 2001, the GMHP saw a 41% increase in attendees at clinics. A designated health advisor for intensive contact tracing, education and health promotion was employed. 20.7% of patients diagnosed with syphilis at St. James's Hospital attended as a result of contact tracing. Serological testing for syphilis was performed in gay venues (bars, saunas, clubs, community events and Gay PRIDE). 896 men tested for syphilis in these venues. 54 new diagnosis of syphilis were confirmed through this method. (75% were diagnosed with early infectious syphilis with a clinical history of primary or secondary syphilis or contact with a known syphilis case) 24% of these men were codiagnosed with at least one other STI; two men were diagnosed with 5 infections (HIV, HBV, *N. gonorrhoea*, *C. trachomatis*, condyloma acuminata). A poster and information campaign targeted at gay men that included images of 'drag queens and kings' was implemented. Peer outreach workers highlighted important syphilis facts in face-to-face interviews with community members.
Conclusion: Intervention measures have proven successful for case finding in the context of this outbreak. The links developed during this outbreak have fostered trust between the gay and bisexual community and health services providing the basis for further collaboration in the future.

PCR and sexually transmitted diseases

P1172 Performance of the APTIMA CT assay for the detection of *Chlamydia trachomatis*

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Objectives: The APTIMA CT assay has been developed to qualitatively detect *Chlamydia trachomatis* rRNA from male urethral, endocervical, and vaginal swab specimens and male and female urine specimens. A target capture method is used to purify the CT nucleic acid away from both nontarget nucleic acids and the amplification inhibitors found in clinical specimens. Following Transcription-Mediated Amplification (TMA), the products of the amplification reaction are then detected by the Hybridization Protection Assay (HPA). The assay was designed to be used as both a confirmatory assay for the CT portion of APTIMA Combo 2 as well as a direct test for CT.
Methods: A panel of 154 bacteria, fungi, parasites and viruses was tested with the APTIMA CT assay to demonstrate analytical specificity, and 15 CT serovars were tested to show analytical sensitivity. *E. coli* and *G. vaginalis* (1×10^8 cells/assay) were added to specimens containing the CT rRNA equivalent of 1 CT IFU/assay to test the impact of nontarget organisms. Naturally occurring and exogeneously derived materials were tested for assay interference and impact on background in the presence and absence of CT at the rRNA equivalent of 1 CT IFU/assay. Negative swab and urine specimens were tested for inhibition by the addition of CT rRNA at the limit of detection.
Results: The panel of 154 bacteria, fungi, parasites and viruses produced all negative results, thereby demonstrating analytical specificity. The testing of the 15 CT serovars showed analytical sensitivity to be as low as 0.01 CT IFU/assay for all 15 serovars. None of the naturally occurring or exogenously derived materials interfered with APTIMA CT performance at all the normal and excessive levels tested, including those known to interfere with other amplified assays. No interference with signal recovery was observed from the nontarget organisms tested. No inhibitory results for any of the patient swab and urine specimens tested or for any of the urine specimens tested from patients with confirmed urinary tract infections (0/200 swabs; 0/285 urine specimens) were observed.
Conclusions: Target capture is effective in removing endogenous and exogenous specimen inhibitors and TMA combined with HPA yields highly sensitive and specific detection of CT rRNA. The APTIMA CT assay is currently under development.

P1173 Ability of the APTIMA Combo 2 assay to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in liquid PAP samples

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Objectives: Nucleic acid amplification (NAA) screening for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) at the time of PAP testing is a proposed strategy for controlling these sexually transmitted infections. Our objectives were to determine the sensitivity of the APTIMA Combo 2 assay to detect CT and GC in fresh and stored liquid PAP samples.
Methods: Varying dilutions of CT and GC rRNA were spiked into Gen-Probe specimen transport media (STM) and two liquid PAP systems: PreservCyt (CyTyc) and Autocyte (TriPath); then assayed for detection sensitivity immediately and after storage. Liquid-prep samples containing only CT or GC were mixed in varying concentrations to determine the ability of the dual detection assay to identify single or dual infections. Cervical swabs were collected from women immediately after an Autocyte sample was collected for a PAP test and both samples were assayed for CT and GC after the PAP test was performed.
Results: The APTIMA Combo 2 CT and GC assay sensitivity curves for organism rRNA diluted in STM, PreservCyt and Autocyte were parallel to one another and became negative between 8 and 9 log dilutions. Bacterial rRNA was detected in both liquid PAP systems when only CT or GC was present or when both were mixed together either in equal concentrations or when one was in 1000-fold concentration greater than the other. In a limited number of patients enrolled to this point, concordance for the detection of CT in STM and Autocyte media have been identical and this clinical component is ongoing.
Conclusion: The APTIMA Combo 2 assay detected low levels of CT and GC from both PreservCyt and Autocyte media with equal efficiency to the assay's STM. Detection was still possible after 3 weeks storage of the specimens. CT was detected in equal numbers of clinical samples of Autocyte or STM collections.

P1174 Simultaneous detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the APTIMA Combo 2 assay by testing cervical swabs previously tested by LCx or ProbeTec assays

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Objectives: Laboratories changing from one commercial test to another require assay-specific collection kits to be replaced in clinics serviced. Equivocal results in one assay may benefit from repeat testing in another. The objective of the study was to test by the APTIMA Combo 2 assay residual cervical swab material left over from LCx and ProbeTec-specific collection tubes; then to calculate the concordance of the assay results for *Chlamydia trachomatis*. *Neisseria gonorrhoeae* nucleic acids were simultaneously assayed in the APTIMA Combo 2 assay.
Methods: Swabs previously tested by the ProbeTec CT assay (Becton Dickinson) ($n = 150$) or LCx CT test (Abbott) ($n = 156$) were retrieved after storage (2 weeks refrigerated or frozen) and tested in the APTIMA Combo 2 assay (Gen-Probe Incorporated) for CT and GC simultaneously. The original swab and 200 µL of the remaining transport media were added to the APTIMA Combo 2 tube before processing.
Results: The APTIMA Combo 2 CT assay results were concordant with the LCx results in 91.4% (32/35) of the positives, 100% (121/121) of the negatives and 98.1% (153/156) overall. APTIMA Combo 2 CT results were concordant with 95.2% (40/42) of positives, 99.1% (107/108) of negatives and 98% (147/150) overall with the ProbeTec CT results. Six patients were found to be dually infected with CT and GC in the APTIMA Combo 2 test. Examination of patient charts revealed that five of the six had been cultured for GC and were positive.
Conclusions: Residual cervical swab material from the LCx and ProbeTec transport systems provided simultaneous NAA testing for CT and GC by APTIMA Combo 2 and identified 93.5% (72/77) of women who had been *C. trachomatis* positive in the other assays. GC-positive patients were also identified.

P1175 Specific detection of *Neisseria gonorrhoeae* by the APTIMA GC assay

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Objectives: The APTIMA GC assay has been developed to allow for the specific capture of *Neisseria gonorrhoeae* rRNA from male urethral, endocervical, and vaginal swab specimens and male and female urine specimens onto magnetic particles as a sample processing method that effectively removes inhibitors of nucleic acid amplification (target capture). GC rRNA is qualitatively detected via Transcription-Mediated Amplification (TMA) and by the Hybridization Protection Assay (HPA). This assay may be used as both a confirmatory assay and as a stand-alone diagnostic assay.
Methods: The analytical performance characteristics of the APTIMA GC assay were evaluated by testing potentially interfering substances such as blood, white blood cells, gynecologic products, and nontarget organisms in endocervical swab specimens and female urine specimens for assay interference and impact on background. Additionally, naturally occurring substances such as protein, glucose, ketones, bilirubin, leukocytes, and nitrite, as well as exogeneously derived materials such as vitamins, minerals, and over-the-counter painkillers were tested in normal urine. To test the impact of nontarget organisms on the assay, *E. coli* and *G. vaginalis* (1×10^8 cells/assay) were added to specimens containing the GC rRNA equivalent of 50 cells/assay. The frequency of specimen inhibition was determined by the

addition of GC rRNA at the limit of detection to negative swab and urine specimens.
Results: No signal recovery or background interference from any of the naturally occurring or exogeneously derived substances was observed at all the normal and excessive levels tested, including those known to interfere with other amplified assays. As well, no interference with assay performance was observed by the tested nontarget organisms. No inhibitory results for any of the patient swab and urine specimens tested for specimen inhibition or for any of the urine specimens tested from patients with confirmed urinary tract infections (0/200 swabs; 0/285 urine specimens) were found.
Conclusions: The APTIMA GC assay is a sensitive and specific assay for detecting GC and target capture effectively removes potentially inhibitory substances. The APTIMA GC assay is currently under development.

P1176 Evaluation of the VIDAS PROBE NG Test for the detection of *Neisseria gonorrhoeae* in female endocervical and vaginal swab and urine specimens

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Objectives: The VIDAS PROBE *Neisseria gonorrhoeae* Test* (VPNG, BioMérieux, Rockland, MA) was evaluated against patient infection status, determined by confirmed endocervical swab culture and APTIMA Combo 2 (AC2, Gen-Probe, Incorporated, San Diego, CA). Specimen types tested included endocervical swabs, self-collected vaginal swabs, and urines.
Methods: A total of 224 patients were evaluated. Testing was performed on 224 endocervical swabs, 223 vaginal swabs, and 214 urines. Specimens were processed for the VPNG and AC2 assays per manufacturers' instructions. VPNG processed specimens were added to the VIDAS PROBE NG strip, amplified on the AMPstation, and detected on the VIDAS. AC2 processed specimens were amplified in a water bath and detected by the LEADER detection instrument. Swabs were plated for *N. gonorrhoeae* detection by culture. A patient was considered positive if confirmed culture or both AC2 swab and urine specimens were positive.
Results: The sensitivity and specificity of the VIDAS PROBE NG Test in endocervical swabs were 100% (28/28) and 97% (188/194), respectively. In vaginal swabs, the sensitivity and specificity were 96% (27/28) and 95% (177/186), respectively. In urines, the sensitivity and specificity were 88% (23/26) and 100% (178/178), respectively. Eight patients were excluded due to negative culture and discordant AC2 results (6 AC2 swab positives and 2 AC2 urine positives). Internal control invalid rates were 0.9% (2/224) for endocervical swabs, 3.1% (7/223) for vaginal swabs, and 4.2% (9/214) for urines. Equivocal rates were 1.3% (3/224) for endocervical swabs, 3.1% (7/223) for vaginal swabs, and 0.5% (1/214) for urines.
Conclusions: The VIDAS PROBE NG Test is sensitive and specific compared with patient infection status in endocervical swabs, vaginal swabs, and urines. The use of an internal control reliably predicts the ability of each assay to amplify target. Low invalid and equivocal rates minimize retests and recollection of samples.

*This product has not been approved by the United States FDA and is not commercially available.

P1177 Evaluation of the VIDAS PROBE NG Test for the detection of *Neisseria gonorrhoeae* in male swab and urine specimens

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Objectives: The VIDAS PROBE *Neisseria gonorrhoeae* Test* (VPNG, BioMérieux, Rockland, MA) was evaluated against patient infection status, determined by confirmed urethral swab culture and APTIMA Combo 2 (AC2, Gen-Probe, Incorporated, San Diego, CA). Specimen types tested included urethral swabs and urines.
Methods: A total of 159 patients were evaluated. Testing was performed on 158 urethral swabs and 156 urines. Specimens were processed for the VPNG and AC2 assays per manufacturers' instructions. VPNG processed specimens were added to the VIDAS PROBE NG strip, amplified on the AMPstation, and detected on the VIDAS. AC2 processed specimens were amplified in a water bath, and detected by the LEADER detection instrument. Swabs were plated for *N. gonorrhoeae* detection by culture. A patient was considered positive if confirmed culture or both AC2 swab and urine specimens were positive.
Results: The sensitivity and specificity of the VIDAS PROBE NG Test in urethral swabs were 98% (39/40) and 99% (116/117), respectively. In urines, the sensitivity and specificity were 97% (38/39) and 100% (115/115), respectively. Internal control invalid rates were 0.6% (1/158) for urethral swabs and 1.3% (2/156) for urines. Equivocal rates were 2.5% (4/158) for urethral swabs and 2.6% (4/156) for urines.
Conclusion: The VIDAS PROBE NG Test is sensitive and specific compared with patient infection status in urethral swabs and urines. No difference in assay performance was observed between swabs and urines. The use of an internal control reliably predicts the ability of each assay to amplify target. Low invalid and equivocal rates minimize retests and recollection of samples.

*This product has not been approved by the United States FDA and is not commercially available.

Clinical and epidemiologic aspects of hepatitis C infection

P1178 The prevalence of hepatic granulomas in chronic hepatitis C

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Objectives: Hepatic granulomas (HGs) are chronic inflammatory lesions composed of modified macrophages – epitheloid cells – that are usually surrounded by lymphocytes. The common etiologies include sarcoidosis and tuberculosis worldwide. Formation of HG is a very rare complication of chronic hepatitis C (CHC) infection. Data about the prevalence of HG in CHC is very limited. The correlation of hepatic granulomas to the response to therapy is controversial. In this study, we aimed to determine the prevalence of HG in CHC, and the relation of HG with interferon treatment.
Patients and methods: All patients with chronic hepatitis C (CHC) in two university hospitals of Istanbul were included into the study. All biopsy reports of the patients were reviewed. The ones which reveal HGs were further studied. Before ascribing hepatic granulomas to CHC, the other causes were excluded: a careful history including a detailed drug questioning, physical examination, biochemistry, tuberculin skin test, VDRL test, Brucella agglutination tests, and thorax CT.
Results: Liver biopsies of 459 patients with CHC were reviewed. Eight patients were found to have hepatic granulomas (8/459, 1.7%). Four patients had control biopsies following a treatment of interferon: hepatic granulomas persisted in all although two had sustained response.
Conclusion: Hepatic granulomas are rarely seen in liver biopsies of the patients with CHC. Data from limited number of patients revealed that HG seems to persist whether the patient responded or not.

P1179 Anti-HBc alone, associated with hepatitis C virus coinfection

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Objectives: Subjects with antibodies against hepatitis B core antigen as the only serologic marker for hepatitis B virus (HBV) infection were found to be often infected with hepatitis C virus (HCV) (Jild et al. Individuals with antibodies against hepatitis B core antigen as the only serological marker for hepatitis B infection: high percentage of carriers of hepatitis B and C virus. J Hepatol 1995; 23: 14–20). The aim of this study was to examine this correlation and to assess the impact of HCV infection on aHBs level.

Methods: In a period of 18 months we examined 4711 samples for hepatitis B markers and anti-HCV antibodies by enzyme immunoassay (EIA) (AxSym, Abbott).
Results: Of the 4711 samples, 1870 (39.7%) were positive for anti HBc antibodies. Of the 1870 samples positive for aHBc, 1031 (55%) had anti-HBs antibodies, 408 (21.8%) were chronic carriers of HBs antigen, while 268 (14.3%) were aHBc-alone-positive subjects. Anti-HBc carriers who also possess anti-HBs or HbsAg found HCV-positive in a number of 85 (5%) while aHBc-alone-positive had anti-HCV antibodies in a number of 24 (14.3%).
Conclusions: We proved that aHBc-alone-positive subjects are more often seropositive for HCV than anti-HBc carriers who also possess anti-HBs or HbsAg. Moreover, HCV-positive subjects with anti-HBs had a lower level of aHBs than HCV-negative subjects.

P1180 Low hepatitis C virus transmission to children in hepatitis C virus and human immunodeficiency virus coinfected mothers on antiretroviral therapy

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Background: HIV has been reported to increase the risk of vertical transmission of HCV in women coinfected. However, an aggressive antiretroviral treatment could be implicated as a factor reducing the rate of HCV transmission.
Objectives: To compare the rate of HCV vertical transmission from mothers with and without concomitant HIV infection and to evaluate the possibly risk factors.
Methods: A total of 642 HCV-reactive (EIA III) mothers has been prospectically observed; 413 were HCV-RNA positive (RT-PCR) with different genotype (genotype 1a, 1b, 2, 3a, 4 and indeterminate in 82, 129, 101, 81, 18 and two patients, respectively) and, of this group, 45 were HIV coinfected on antiretroviral therapy (18 on double regimen, 27 on triple regimen). The rate of HCV transmission and the related risk factors were evaluated in this HIV+/HCV+ coinfected women and compared with the 368 viremic HCV+/HIV− mothers. AST, ALT, anti-HCV Ab, HCV-RNA, anti-HIV Ab (EIA), and HIV-RNA (RT-PCR) of infants were obtained at birth and during the 24 months follow-up. HCV genotyping were determined by Genedia KIT.
Results: One case of HCV infection and one case of HIV infection were observed in infants born to coinfected mothers vs. 14 cases of HCV infected infants born to anti HCV+/HIV− mothers: no difference in the risk of vertical HCV transmission has been observed in the two groups of childbearing women (Fischer exact: two-tailed *P*-value 1.0). Difference was observed in the distribution of the HCV genotypes (1a in 18 and 64, 1b in 13 and 116, 2 in three and 98, 3a in eight and 73, 4 in one and 17, respectively), reflecting the high proportion of intravenous heroin addicts in the HIV+ women. The maternal HCV-RNA titer was similar (2 466 090 ± SD 1 098 900 copies/mL vs. 2 051 000 ± SD 1 121 000 copies/mL, respectively). Delivery was by means of Cesarean section in 35 (78%) of the HIV positive women vs. 93 (25%) of the HCV+/HIV− mothers. Among the HCV+/HIV+ coinfected mothers the HIV-RNA level was below the limit of detection in 33 cases and low in the remaining 12. No HIV-positive women were breast feeding.
Conclusion: An effective antiretroviral therapy could prevent HCV mothers to child transmission in HCV viremic HIV coinfected women, possibly via a control of the HIV viral load and the consequent immunoriconstitution. Type of delivery could be a cofactor in preventing vertical HCV infection.

P1181 Risk factors in mother-to-child hepatitis C virus transmission

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Background: Vertical transmission of hepatitis C virus (HCV) has been estimated to be low (0–5%) in women with HCV alone and 5–15% in women coinfected with human immunodeficiency virus (HIV). The aim of this study was to evaluate a cohort of children born to HCV infected women with particular emphasis on the role of risk factors in parents and HCV serologic progress in children.
Subjects and methods: The study included 67 children born to mothers with HCV infection. Screening for HCV antibody was carried out on the serum samples using a third-generation enzyme-linked immunoassay and confirmed by a third-generation line immunoblot assay. The detection of serum HCV-RNA was done by using reverse transcription polymerase chain reaction with primers from the 5′ noncoding region of the viral genome. For statistical analysis, chi test was used, being $P < 0.05$ statistically significant.
Results: The risk factors for HCV infection in the progenitors were: unknown in 33 cases (49.25%), past or current intravenous drug user in 33 cases (49.25%) and past blood transfusion in one case (1.49%). HBsAg was detected in six progenitors, anti-HIV in 23, HBV and HIV coinfection in five and there was no coinfection in 33 cases. With respect to mothers treatment, 43 were not treated at the time of pregnancy, five mothers with HIV coinfection were treated with zidovudine, eight intravenous drug users were treated with methadone and 11 were treated with interferon. Forty-four children were no coinfected with other viruses and 23 (34.23%) were coinfected, principally with HIV (19 of them). At birth, there were 24 (35.82%) anti-HCV positive and HCV-RNA positive and 43 (64.17%) children anti-HCV positive and HCV-RNA negative. During the time, there was a seroreversion and at 12 months, there were 44 (75.86%) children anti-HCV positive and HCV-RNA negative and 14 (24.13%) anti-HCV positive and HCV-RNA positive.
Conclusion: There was a strong interaction between intravenous drug user and delivery of HCV infection. However, when the progenitors were intravenous drug users there was a strong interaction between progenitors risk factors for HCV infection and child coinfection with other viruses, in particular HIV. On the other hand, mothers treatments did not interfere in the child infection progress. There was no association in children between birth analysis and progress of HCV antibodies.

P1182 Genotype distribution in chronic hepatitis C patients in Luxembourg

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Objectives: HCV genotype is associated with response to treatment. The HCV genotype distribution was studied in chronic hepatitis C patients in Luxembourg. Possible associations with age, gender and ways of contamination were investigated.
Methods: The genotype and subtype were determined in RNA-positive EDTA samples from 234 HCV infected patients by direct sequencing of the 5′ NC gene fragment (TruGene HCV 5′ NC genotyping kit, Bayer).
Results: One hundred and twenty-nine patients were men (62%) and 125 patients were older than 40 years (53%). Of the 234 samples, 75 (32%) could only be characterized to the genotype level (no unambiguous subtype determination). Genotype 1, subtypes a and b in equal proportions, was present in 135 patients (58%) with no difference between gender. Genotype 3, mainly subtype a, was present in 63 patients (27%), 45 men (32%) and 18 women (20%). Genotype 2 and 4 were less common, with 18 cases of genotype 4 (8%), mostly with undetermined subtype and 15 cases of genotype 2 (6%), subtypes a and b. Three samples of genotype 5 were also found. Genotype 3 was more frequent in patients younger than 40 years (32%) compared with older patients (22%) but without reaching significance ($P = 0.09$). Information about possible ways of contamination was available for 143 patients. Genotype 3 was significantly ($P < 0001$) more prevalent among IVDU ($39/94 = 41.5\%$) compared with other risk groups ($7/49 = 14.3\%$).
Conclusions: Genotypes 1 and 3 represent together 85% of chronic hepatitis C patients in Luxembourg. Genotype 3 was especially prevalent in IVDU, pointing to a possible epidemiologic link in these patients. Genotype 3 patients tended also to be younger and were more often men. Genotype 1 and 4, associated with a poorer treatment response, represent two-thirds of all patients.

P1183 Prevalence of hepatitis C virus infection in cancer patients. A 3-year study

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Objectives: The aim of our study was to investigate the prevalence of hepatitis C virus (HCV) infection in cancer patients, since they constitute a high risk group.

Methods: During the 3-year period (2000–2002) serum samples were taken from 1579 hospitalized patients with cancer and were evaluated for the presence of the antibody against HCV. The detection of the antibody to HCV in the serum of cancer patients was made by the ELISA method (Microparticle Enzyme Immunoassay MEIA-AXSYM, ABBOTT).
Results: Of the 1579 cancer patients 48 (3.04%) were positive for the antibody against HCV. The prevalence of HCV infection per year for 2000, 2001 and 2002 was 3.1% (18 out of 580 patients), 3.09% (17 out of 549 patients) and 2.88% (13 out of 450 patients), respectively.
Conclusions: Our study shows that the frequency of the antibody to HCV in the serum of cancer patients during the years 2000–2002 was 3.04% and that the prevalence of HCV infection in cancer patients was significant and remained roughly the same per year.

P1184 Evaluation of total HCV core antigen detection in follow-up of children born to HCV positive mothers

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Objectives: To propose an immunoenzymatic antigenic detection test for early diagnosis of mother–fetus HCV infection transmission.
Methods: The work was carried out on the born to HCV positive mothers group, followed-up by means of the Track-C experimental test (Ortho Clinical Diagnostic) on scheduled blood samples collected between birth and 18 months, in the period January 1, 2001–December 31, 2002. One hundred and thirteen sera belonging to 80 children born to HCV positive mothers with different antibody titers, were tested. The routine follow-up was carried out by means of a qualitative HCV PCR and HCV antibodies quantitative test at birth, 6th week, 3rd, 6th, 9th, 12th and 18th months (when in most cases maternal antibodies disappear) according to little patients availability. Track-C-test was performed on frozen selected sera already tested with an immunometric technique (Ortho Vitros ECi) for HCV antibodies and Roche Cobas Amplicor for qualitative PCR.
Results: One hundred and five sera belonging to 75 children were negative for HCV core antigen with 100% agreement with PCR. Eight consecutive samples of five children were positive for HCV core antigen and all were then confirmed by PCR test. Consecutive PCR test were performed on sera of HCV antigen negative children and gave negative results.
Conclusions: The specificity of HCV core antigen can be estimated as 100%; the diagnostic sensitivity is probably good but cannot be quantitatively assessed yet. This test certainly does not need special equipment and facilities, is easy to perform and cheaper than molecular test. Furthermore this antigenic detection test shown to be useful in early diagnosis of HCV infection in our study group.

P1185 Analysis of peripheral T-lymphocyte subsets and antibody responses in patients with chronic HCV-infection

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Objectives: The aim of this study was to investigate the peripheral blood lymphocyte phenotypes (T-cells population) and quantitative antibody response (general anti-HCV and anti-HCV response to structural (core) and nonstructural (NS3, NS4, NS5) hepatitis C viral proteins) in patients with different stages and different activity of chronic HCV-infection (chronic hepatitis C, HCV-RNA+/–, HCV-liver cirrhosis).
Methods: Blood lymphocytes counts were determined in 36 patients with chronic hepatitis C (CHC) and 16 patients with liver cirrhosis (LC). Flow cytometry ('Becton Dickinson', USA) was used to measure proportion of T cells (CD3+, CD4+, CD8+, CD16+/56+, CD3/16+). Anti-HCV antibodies investigated by third-generation enzyme linked immunosorbent assay, HCV-RNA presence in serum was determined by using PCR. Relative intensity (RI) of antibody titer for each patient was calculated comparatively with normal (lower limit of the assay) in every series of the researches.
Results: The peripheral blood lymphocytes of patients with CHC contained a greater proportion of CD8+ (28.11 ± 3.61 vs. 22.28 ± 2.8, $P < 0.01$) lymphocytes than patients with HCV-LC. In individuals with liver cirrhosis significantly higher median antibody responses to core (12.41 RI vs. 8.27 RI, $P = 0012$) were found comparatively to chronic hepatitis C patients. Significantly higher proportion of CD16/56+ (NK) cells ($12.73 \pm 3.99\%$ vs. $7.17 \pm 2.85\%$, $P < 0.05$) were found in the individuals with persistent viremia (HCV-RNA+) than in those with apparent resolution of HCV RNA (HCV-RNA) in the blood. In the group of patients with elevated ALT activity higher median antibody titer to core protein (9.26 ± 4.98 RI vs. 13.73 ± 5.76 RI) and decrease of median NS3, NS4, NS5 antibody titers (3.69 ± 2.39 vs. 7.0 ± 3.63) were detected comparatively to patients with normal ALT level, but this difference was not significant ($P > 0.05$) in both cases. Inverse correlation was estimated between the percentage of CD16/56+ cells and RI of HCV-antibody response (Spearman range correlation $= -0.669$, $P < 0.01$).
Conclusions: Increased percentage of CD16/56+ (NK) cells was associated with HCV-RNA viremia and could be a marker of virus persistention in patients with chronic hepatitis C. The antibody response to core protein was higher in patients with advanced liver disease and patients group with elevated ALT activity.

P1186 Seroprevalence of antibodies to hepatitis C virus among hemodialysis patients

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Introduction: Chronic liver disease represents one of the most important causes of morbidity and mortality among hemodialysis patients, hepatitis C viral infection being mainly responsible for these affections.
Objectives and methods: The aim of our study is to evaluate the prevalence of hepatitis C infection (ELISA-Abbot anti HCV antibodies test) in 52 patients with chronic renal failure treated in the hemodialysis department of our hospital. We have also analyzed the relationship between hepatitis C infection and blood transfusion history among these patients. The age of patients was between 23 and 70 years. According to sex, 31 patients were male, and the other 21 were female.
Results: Antibodies against hepatitis C virus were detected in 10 patients (prevalence 19%), six male, and four female. Among the positive patients, four had received blood transfusion. Among the other 42 patients, 19 had a blood transfusion history (45%), but they were not infected by the hepatitis C virus. The prevalence of virus C infection in patients without a blood transfusion history (10.55%) is greater then among those who had received blood transfusion (4.5%). We have analyzed the relationship between virus C infection and previous blood transfusion and we have not found significant statistical differences between the groups of our patients.
Conclusions: The prevalence obtained among our hemodialysis patients is similar to prevalence described in other hemodialysis populations. We have not found blood transfusion history as an only risk factor of virus C infection among our hemodialysis patients.

P1187 Hepatitis C in rheumatoid arthritis patients

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Objectives: Hepatitis C virus (HCV) infection has been explained as sometimes presenting with rheumatic manifestations indistinguishable from rheumatoid arthritis. In some studies hepatitis C virus is supposed to be able to trigger rheumatoid arthritis. So this study has been performed to evaluate the frequency of hepatitis C virus infection in a group of patients with rheumatoid arthritis.
Methods: In this study the serum of one hundred consecutive patients with rheumatoid arthritis from all affiliated hospitals of Shaheed Beheshti University of Medical Sciences during 1 year (2000) were examined for anti-HCV antibody by ELISA and HCV-RNA by RT-PCR method. Using a questionnaire, the frequency of HCV infection, the age and the sex distribution, duration of rheumatoid arthritis, associated immune mediated disorders and risk factors for hepatitis C virus infection were defined.
Results: A total of 100 patients (M/F = 1) who were mainly in the age group of 31–50 years was studied. The frequency of HCV was found to be 2%. All of the infected persons have had a low risk occupation in terms of exposure to the virus and none of them had HCV risk factors. No associated immune mediated disorder was found in HCV infected patients.
Conclusions: This study does not support any contribution of HCV infection in the pathogenesis of rheumatoid arthritis.

P1188 Virological features in adult beta-thalassemic patients with chronic HCV infection

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Objectives: To compare viral load and genotype distribution of adult beta-thalassemic patients to those of nonthalassemic HCV-infected patients.
Methods: Sixty-seven adult beta-thalassemic patients (27 males, 40 females, mean age 30.5 ± 7.3 years) and 49 otherwise normal patients (33 males, 16 females, mean age 31.4 ± 7.4 years) with chronic HCV infection were evaluated. All the patients were HCVAb (+) by MEIA HCV 3.0 (Axsym-Abbott) and by the confirmatory linked immunoassay (Sorin). The patients were tested for HCV RNA by RT-PCR (Amplicor HCV PCR test). Quantification of serum HCV RNA was performed by the branched DNA method (Bayer HCV RNA 3.0 Assay). HCV genotyping was performed by a second generation, line probe assay (INNO-LiPA HCV II, Innogenetics).
Results: HCV RNA was detected in 41/67 (61.2%) beta-thalassemic and in 41/49 (83.7%) nonthalassemic patients ($P = 0.009$). Serum HCV RNA levels, were lower in the thalassemic group (median = 0.364 Meq/mL, range = 15.297 Meq/mL) than in the nonthalassemic group (median = 2.900 Meq/mL, range = 54.780 Meq/mL, $P = 0.009$). The distribution of HCV genotypes was different in the two groups ($P = 0.001$). In beta-thalassemic patients the most prevalent HCV genotype was 4 (11/34 = 32.4%) while in nonthalassemic patients it was 3a (25/41 = 61%).
Conclusions: These data indicate that (1) thalassemias have a higher rate of elimination of the virus, probably because of acquiring the infection during the childhood, (2) HCV viral load is lower in adult beta-thalassemic patients compared with that of nonthalassemic patients with chronic hepatitis C, and (3) the most prevalent genotype in Greek adult beta-thalassemic patients is genotype 4 while in nonthalassemic patients of similar age it is genotype 3a. This difference is probably attributable to the different way of virus transmission in the two populations of patients.

P1189 Prevalence of cryoglobulinemia in adult beta-thalassemic patients with chronic HCV infection

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Objectives: To determine the prevalence of cryoglobulinemia in adult beta-thalassemic Greek patients with chronic HCV infection.
Methods: Thirty-one HCVAb(+) adult beta-thalassemic patients (11 males, 20 females, mean age 31.4 ± 6.8 years) and 49 otherwise normal patients (33 males, 16 females, mean age 31.4 ± 7.4 years) with chronic HCV infection were evaluated. Serum HCV RNA was detected by RT-PCR. The presence of cryoglobulins was assayed, by collection and coagulation of blood at 37 °C, centrifugation and incubation of serum at 4 °C for 7 days. Rheumatoid factor (RF) and antinuclear (ANA) were also detected in serum.
Results: Cryoglobulins were detected in 2/29 (6.5%) beta-thalassemic and in 10/39 (20.4%) nonthalassemic patients ($P = 0.089$, NS). HCV RNA was detected in 20/31 (64.5%) beta-thalassemic and in 41/49 (83.7%) nonthalassemic patients ($P = 0.05$). When we compared the prevalence of cryoglobulinemia between thalassemic and nonthalassemic HCV RNA(+) patients, the difference was also not found significant (2/20 = 10% vs. 10/41 = 24.4%, NS). Positivity rate for rheumatoid factor was found lower in the beta-thalassemic group (2/31 = 6.5%) than in the nonthalassemic group (15/49 = 30.6%, $P = 0.01$). ANAs were found with equal frequency in the two groups (4/31 = 12.9% vs. 3/49 = 6.1%, NS). There was no association between the presence of cryoglobulins and patients' age or sex.
Conclusion: Prevalence of cryoglobulinemia was found low (6.5%) in beta-thalassemic HCV-infected patients although not significantly different from that found in nonthalassemic patients. This low prevalence can be attributed to: (1) the lower rate of HCV RNA positivity that was found in these patients and (2) the immunosupression that is induced by multiple transfusions in beta-thalassemic patients.

Herpesvirus

P1190 Possible risk factors for the triggering of the human herpesvirus 8 in two non-HIV patients

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We report our experience originated from two patients with Kaposi's sarcoma, although they were human immunodeficiency virus negative. The first patient was a 70-year-old-heterosexual man who referred to our clinic with painful disseminated skin lesions on the limbs, face and abdomen. Dermatological examination revealed painful hard nodules and infiltrates that were red violent to brown without tendency to ulceration. Later similar lesions on the both upper and lower eyelid margin developed. Extensive and multiple laboratory tests, computed tomograghy of the chest and abdomen rule out infection or neoplastic diseases but did not elucidate the patient's illness. According to the past medical history, 5 months ago due to the patient's chest radiographic features were very consistent with interstitial lung disease possibly due to collagen vascular disorder he was placed on methylprednizolone 16 mg twice daily for almost 4 months. Biopsy excision of the skin lesions was performed and the histopathological picture suggested Kaposi's sarcoma. Peripheral blood mononuclear cells were tested positive for Human Herpesvirus 8 (HHV 8) by PCR and immunohistochemistry of the tissue. The second patient was a 26-year-old heterosexual woman who presented to our hospital with lesions that first appeared on the left feet and on the right knee. Several violaceous lesions were later observed on the arms, hands and face. Severe bilateral lymphedema of the legs with a reddish papules nodules and tumors from 0.5 to 7 cm in diameter on the soles, toes and calves was also observed. The patient among the other laboratory and radiographic test underwent thoracic CT scan, which demonstrated mediastinal well defined soft tissue infiltration associated with mediastinitis and a well defined mass in the left paratracheal region. The mass biopsy revealed squamous cell carcinoma of the esophagus. Peripheral blood mononuclear cells were tested positive for HHV 8 by PCR. Immunosuppression induced by either corticosteroid or carcinoma is a risk factor for the triggering of the HHV 8 and the development of KS, probably in genetically susceptible patients. Review of the literature revealed that these are almost two very rare cases of Kaposi's sarcoma induced from corticosteroid (reported about 10 cases) or induced from a carcinoma (reported about four cases).

P1191 Cytomegalovirus congenital infection: evaluation of different diagnostic procedures

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Objectives: Human cytomegalovirus (HCMV) represents the most dangerous microorganism for the newborn. The virus can be transmitted to the fetus, causing severe consequences on his development. Different approaches are observed in the management of this situation. The aim of this study was to evaluate the different diagnostic procedures performed in order to detect primary infection in pregnant woman and congenital infection in fetus and newborn.
Methods: Fifty-two pregnant women and their newborns were evaluated from 1996 to 2002 for suspected HCMV infection. Specific IgG, IgM and IgG avidity were detected in maternal serum samples at different times during pregnancy and in the newborns at birth. A total of 22 amniotic fluids, 12 placentas, 24 maternal and 34 neonatal blood samples, 37 urine and 32 saliva samples from neonates were assayed by nested PCR for detection of viral DNA.
Results: Ten newborns with congenital infection were identified, seven asymptomatic at birth and three symptomatic (thrombocytopenia, anemia and cerebral calcifications with viral DNA in cerebrospinal fluid, respectively). Twenty two seroconversions were documented: 10 in the first, 10 in the second and two in the third trimester of pregnancy, but the IgG avidity test could identify 32 acute primary infection. Eight out of 10 congenital infections occurred in presence of low avidity IgG at serologic screening and in the other two in absence of data about the serological pattern during the

course of pregnancy. Prenatal diagnosis showed viral DNA at different amount in three amniotic fluid samples (one asymptomatic congenital infection, one pregnancy interrupted and one still in course). One false negative result was reported. Two placentas contained HCMV-DNA: the newborns were infected. Maternal blood resulted PCR positive in only one woman, who had spontaneous abortion. Urine and saliva samples provided viral DNA in all congenital infections.
Conclusions: HCMV is the leading cause of congenital infection, but, despite the availability of several serologic and molecular tools, the diagnosis in pregnant women is still difficult. Furthermore, maternal and fetal prognostic markers of infection and disease are still unidentified. Interdisciplinary collaboration should be encouraged to promote standardized guidelines for an optimal management of this relevant public health problem.

P1192 Epstein-Barr virus PCR in serum is not sufficient to detect early acute infection

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Objectives: In acute Epstein-Barr virus (EBV) infection, serology may not be conclusive if samples are obtained at an early stage. The aim was to determine whether EBV-PCR can detect early EBV infection when serology is inconclusive.
Methods: One hundred and thirteen sera were classified as early acute (46), late acute (36), and past infection (29) on the basis of EA IgG, EA IgM, and EBNA-1 IgG by EIA (Biotest, D-Dreieich) and on recombinant IgG and IgM immunoblot (EA p54/p138, VCAp18/p23, EBNA1; Mikrogen, D-Martinsried), heterophile antibodies, white blood count, and transaminases where available. Following extraction of DNA by the MagnaPure Total Nucleic Acid Isolation protocol, PCR was performed on the LightCycler system using the Epstein-Barr Virus Kit (Roche, CH-Rotkreuz). The limit of detection was 10 viral genome equivalents per PCR.
Results: Nineteen of 46 (41.3%) early acute, 12 of 36 (33.3%) late acute, and one of 29 (3.4%) past infection were PCR-positive.
Conclusions: With a sensitivity of only 37.8% (CI95 28.1–48.6) in acute stages of EBV infection, PCR from serum samples was not helpful for diagnosis. The reason may be the close cellular association of the virus which may result in low numbers of freely circulating viral DNA. As only one of 29 cases of past infection was PCR-positive (specificity 96.6%; CI95 82.8–99.8), latent EBV infection did not interfere. Thus, serum PCR for EBV does not allow for reliable detection of early acute infection when serology is still inconclusive. Further studies are required to assess the diagnostic value of EBV-PCR in leucocytes from full blood samples.

P1193 Development of a quantitative LightCycler assay for the detection of Epstein-Barr virus DNA

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Background: Epstein-Barr virus (EBV), one of the eight human herpesviruses (HHV4), is a double-stranded DNA virus of ubiquitous spread. The virus is transmitted by salivary contact and most often individuals become infected during their young childhood. In these cases primary infections are mostly asymptomatic or reveal only mild unspecific symptoms. When infection with EBV occurs during adolescence or young adulthood, it causes infectious mononucleosis in up to 50% of all cases, an illness associated with fever and swollen lymph glands. In some cases EBV is described as the causative agent in chronic fatigue syndrome. Worldwide, EBV has a prevalence of about 90%. Once acquired, the virus establishes a lifelong latent infection and remains in epithelial cells of the throat and in B-lymphocytes. There and in very rare events, EBV can cause severe complications like Burkitt's lymphoma or nasopharyngeal carcinoma mainly known from Africans or Asians, respectively. Normally, EBV is under the control of T cells, which prevent their outgrow in healthy individuals. Serious complication may occur after organ or bone marrow transplantations in patients under immunosuppressive therapy. There, EBV can be re-activated leading to a severe post-transplant lymphoproliferative disorder (PTLD) which can have a fatal progression if not diagnosed early.
Methods: Here, we describe the development of a highly sensitive and quantitative PCR assay for research use based on the LightCycler instrument.
Results: This PCR test directly measure the viral DNA load in either plasma, whole blood or CSF. The LightCycler EBV Quantification Kit has been proven as a fast and reliable tool. It offers a sensitivity of less than 10 copies/reaction and gives a linear amplification of at least 5 logs (102–106). The reliability of the complete Nucleic Acid Detection System is granted by the coamplification of an internal control, to exclude false negative results by inhibition. The assay is fully compatible with the PCR Workflow System (MagNA Pure LC Instrument and LightCycler instrument), so enabling complete automation of EBV quantification.
Conclusions: The described EBV assay has a high sensitivity and specificity. The wide dynamic range of the assay make it perfectly suited for a wide variety of research applications.

P1194 Lymphotropic herpesviruses infection in atherosclerotic coronary arteries tissue

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Objectives: To investigate the association of CMV, HHV-6, HHV-7, EBV and HHV-8 with atherosclerosis.
Methods: The presence of beta- and gamma-herpesviruses DNA was investigated by nested PCR in atherosclerotic lesion and unchanged site of vessel taken postmortem from the same person. Also the level of anti-CMV IgG antibodies in serum samples was evaluated.
Results: Atherosclerotic changes evaluated by postmortem examination with 5 point scale were observed in 37 out of 43 investigated persons. Intensity of changes was correlated with age. Antibodies for CMV at different level (from 0.528 to 14.042 IU/mL) were detected in 81.1% cases. CMV DNA in vessels was found in 27.9% persons with atherosclerotic changes, but was not in arterial walls of patients without atherosclerosis (grade 0). Prevalence of HHV-6 and HHV-7 DNA was even higher: 81.4 and 51.2%, respectively, but while the presence of CMV DNA was connected with grade of changes in arteries it seems that HHV-6 and -7 distribution was not dependent on range of atherosclerosis. Distribution of EBV and HHV-8 was lower than other tested herpesviruses.
Conclusions: It will be suggested that high prevalence of different herpesviruses in wall of coronary arteries may be related with the pathologic process of the atherosclerosis.

P1195 Quantitative analysis of Epstein-Barr virus DNA in patients with breast carcinoma

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Background: EBV has been linked with a number of human malignancies, including African Burkitt's lymphoma, classical Hodgkin's disease, post-transplant and acquired immune deficiency syndrome-associated lymphoproliferative disorders, sinonasal NK/T cell lymphoma, nasopharyngeal carcinoma and gastric carcinoma. In recent years, it has been questioned whether EBV may play a role in the development of breast carcinoma (BC), which is the most common malignant tumor and the leading cause of cancer in women in Western countries.
Methods: In an attempt to resolve this question, we studied the incidence of EBV infection in 76 cases of invasive breast carcinoma by using a real-time quantitative PCR assay for EBV DNA (Brengel-Pesce et al. J Med Virol 2002) in order to analyze the viral burden both in peripheral blood mononuclear cells (PBMC) and in tumor cells from BC patients. Immunochemistry using anti-LMP-1 and anti-ZEBRA monoclonal antibodies was also evaluated. Finally, we performed the titration of anti-ZEBRA antibodies in serum to measure the level of EBV replication.
Results: EBV DNA was detected in tumor from 16/33 patients with breast carcinoma and among positive samples the level ranged from 9 to 280 copies per μg DNA. EBV content in PBMC samples ranged from 6 to 250 copies per μg DNA but no correlation was found between the viral burden in PBMC and that in tumor. No EBV genomes were found in the serum but there was a positive correlation between the EBV load in PBMC and increased IgG anti-ZEBRA titers, indicating that a high viral replication occurred in blood.
Conclusion: Overall, our results strength the idea of an association between breast carcinoma and EBV. The lack of EBV detection in serum the absence of correlation between EBV loads in blood and those in tumor might suggest a peculiar role of this oncogenic virus in the pathological process.

Resistant nosocomial pathogens

P1196 Clinical outcome of the vancomycin-resistant *Enterococcus* bacteremia in neutropenic patients with hematologic malignancies – analysis of 22 cases

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Introduction: Infection remains the most frequent cause of morbidity and mortality in neutropenic patients with hematologic malignancies. Bacteremia due to vancomycin-resistant enterococci (VRE) has been characterized as a disease of severely debilitated patients, but it is not sure weather vancomycin resistance is an independent predictor of death. We analyzed 22 patients with hematologic malignancies to assess the clinical pattern of VRE bacteremia in neutropenic patients.
Methods: We conducted a retrospective study of patients diagnosed as having VRE bacteremia, treated at the Department of Hematology. We identified all patients from whom at least two blood culture were positive. We analyzed cases from 2000 to 2002. Antibiotic susceptibility of isolates from blood was determined by disk diffusion method and with reference to standard breakpoints. The primary outcome measure was the in hospital mortality. Polymicrobial infection was defined by isolation of a second bacterial species in a blood culture on the index date.
Results: Among all 22 patients, 1 day positive blood culture for VRE was found in 14 patients, in eight patients a positive blood culture was present at least for 2 days (mean 3 days, min. 2 days, max. 5 days). All patients with VRE bacteremia developed sepsis or SIRS (one patient), however, mortality rate was 9%. We observed 25% polymicrobial blood cultures. All patients were in neutropenia at the time of VRE bacteremia. All patients were treated with imipenem plus aminoglycoside and/or vancomycin. Only three cases were treated with linezolid or quinupristine/dalfopristine (Q/D). We did not observe death in the group of patients treated with Q/D or linezolid. In the group of the patients without linezolid or Q/D treatment, symptoms of sepsis decreased when number of neutrophiles increased.
Conclusions: VRE bacteremia in neutropenic patients is not often associated with severe outcome, mortality rate was about 9%. Good outcome of the neutropenic patients could be improved by new antibiotics, active against VRE, such as linezolid or Q/D.

P1197 Linezolid resistance in clinical isolates of enterococci: risk factors, outcomes, and mechanism

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Objectives: Present an update of our ongoing study to determine risk factors, outcomes and mechanisms associated with linezolid resistance in clinical isolates of enterococci in a tertiary medical center.
Methods: We performed a retrospective chart review of patients receiving linezolid for 72 h or longer for enterococcal infection. All enterococcal isolates were tested for linezolid susceptibility using *E*-test (AB Biodisk). Chromosomal DNA from linezolid-resistant isolates underwent PFGE, and the mechanism of resistance was studied by sequencing ribosomal RNA by a PCR-based method.
Results: Among 88 patients receiving linezolid for more than 72 h for VRE infections, resistant organisms were recovered from 13 (14.8%); 11 were *E. faecium* and two *E. faecalis*. Many of these were associated with treatment failure. Most patients (80%) were in an ICU. In the first 34 patient charts that have been reviewed in detail, duration of therapy (38 days for resistant organisms) was the major risk factor for development of resistance (Pai et al. Clin Infect Dis 2002; 35: 1269). PFGE showed that most isolates emerged during therapy, as only two isolates were identical, which is consistent with nosocomial spread. Sequencing of ribosomal RNA in the first seven resistant isolates revealed the characteristic G2576U mutation in all. Some isolates demonstrated a stepwise increase in MIC, which correlated with the number of ribosomal copies mutated. Linezolid-resistant organisms were cross-resistant to other investigational oxazolidinones but susceptible to quinopristin-dalfopristin, daptomycin, and oritavancin.
Conclusions: Emergence of resistance to linezolid during therapy of enterococcal infections occurs relatively frequently in critically ill patients in our center and is often associated with treatment failure. The duration of therapy is the major risk factor in these patients. Most cases represent true emergence of resistance during therapy. All isolates tested to date have the G2676U mutation. Serial increases in MIC are due to a gene dosage effect. These organisms are resistant to the class of oxazolidinones but susceptible to other newer gram positive agents.

P1198 Analysis of carbapenem-resistant *Pseudomonas* aeruginosa, *Acinetobacter baumanii* and *Klebsiella pneumoniae* for the production of metallo-beta-lactamases

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Objective: Carbapenemases are beta-lactamases able to hydrolyze carbapenems. Most of them also attack other beta-lactam antibiotics. Metallo-beta-lactamases are acquired carbapenemases with significantly increased clinical relevance during the last few years. The aim of this retrospective study was to examine whether Gram(–) bacteria carrying these metalloenzymes had spread in our hospital.
Methods: Between May 2000 and December 2002 a total of 50 clinical isolates of imipenem-resistant *P. aeruginosa* (32), imipenem-resistant *A. baumanii* (12) and imipenem-resistant *K. pneumoniae* (six) were collected from patients either infected or colonized in our hospital. All these isolates were identified by the API20E System (Biomerieux, France). Antibiotic susceptibility testing was performed by the Kirby–Bauer method according to NCCLS guidelines. Imipenem MICs and detection of production of metallo-beta-lactamases were determined by the *E*-test MBL (AB-Biodisk, Solna, Sweden).
Results: The great majority of all isolates were moderate to high level resistant to imipenem (imipenem MICs ranged from 24 to 256 μg/mL). The majority of imipenem-resistant *P. aeruginosa* and all imipenem-resistant *A. baumanii* and *K. pneumoniae* were multiresistant as shown by antibiotic susceptibility testing. Nine of 32 imipenem-resistant *P. aeruginosa*, 7/12 imipenem-resistant *A. baumanii* and 6/6 imipenem-resistant *K. pneumoniae* were found to be MBL producers. All MBL + isolates were also multidrug resistant. MBL + *P. aeruginosa* were isolated from patients in different wards in contrast with MBL + *A. baumanii* and *K. pneumoniae* who were isolated from patients in the ICU.
Conclusions: Our findings indicate that MBL producers are a fact in the hospital environment. MBL + *A. baumanii* and MBL + *K. pneumoniae* seem to still be confined in the ICU environment. Increased awareness of clinicians and laboratory personnel is critical for the control of this resistant pathogen.

P1199 Vancomycin-resistant *Enterococcus faecium* isolated from clinical specimens during a 5-year period

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Objectives: To present the isolation and resistance phenotypes of vancomycin-resistant *Enterococcus faecium* (VREM) isolated from patients hospitalized in Public Hospital No. 1 in Gdansk (Poland) during a 5-year period (1997–2001). Vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens. A significant risk factor associated with acquisition of VRE is the use of cephalosporins and glicopeptides.
Methods: Clinical specimens obtained from hospitalized patients were inoculated on appropriate media. Enterococci were identified by conventional methods (Gram stain, catalase, growth in the presence of 40% bile and 6.5% NaCl, esculin hydrolysis) and by automated system Vitek (bioMérieux). The susceptibility to antibiotics was determined by the disk diffusion method according to the NCCLS guidelines. MICs were determined by *E*-test (AB BIODISK).
Results: During a 5-year period (1997–2001), 253 clinical isolates of VREM were collected from hospitalized patients. Strains were isolated from stool 156 (61.9%), blood 46 (18.2%), urine 16 (6.3%), pus 8 (3.1%) and other specimens 26 (10.3%). Most of the patients with VREM colonization and/or infection were hospitalized in the Hematology Unit. All VREM strains had MIC > 256 mg/L to vancomycin and MIC > 16 mg/L to teicoplanin, which

characterizes phenotype VanA. All VREM were sensitive to chloramphenicol and quinupristin/dalfopristin.
Conclusions: Vancomycin-resistant *Enterococcus faecium* have become an increasing problem in our patients. Further studies to investigate the epidemiology and the resistance of VREM strains in our hospital are warranted.

P1200 MRSA epidemiology in Scotland (1997–2002) – a reference laboratory perspective

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Background: The Scottish MRSA Reference Laboratory (SMRSARL) was established in April 1997. Since then, over 40 000 MRSA isolates have been referred to the SMRSARL from 35 laboratories throughout Scotland. Referrals increased from 4078 in 1997 to a high of 9622 in 2000, decreasing to 8004 in 2002.
Methods: All MRSA referred between 1997 and 2001 were typed by a combination of phenotype, biotype and phage typing. Phage typing was replaced with a molecular PCR-ribotyping technique. Pulsed Field Gel Electrophoresis is carried out on 32% of referrals (18% in 1997). A mutiplex PCR (nuc and mecA gene) is carried out for MRSA confirmation.
Results: Although EMRSA-15 (Sequence Type (ST) 22) still predominates (75% in 1997, 70% in 2002), the percentage of EMRSA-16 (ST 36) isolates has increased (15% in 1997, 25% in 2002). 30 other PFGE defined MRSA clones have been recognized in Scotland, two of which predominate (SMRSA-105 (ST5) and SMRSA-108 (MLST Clonal Complex 8)). Nearly all EMRSA-15 and EMRSA-16 were ciprofloxacin-resistant and most EMRSA-16 were kanamycin-resistant. An increase in EMRSA-15 with gentamicin resistance has been noted in the past 18 months. Also in 2002 an increase of EMRSA-16 with gentamicin and high-level mupirocin resistance emerged. All referrals are screened for intermediate resistant to vancomycin (VISA). Of 10 000 isolates screened for vancomycin intermediate resistance 233 (2%) were positive on first screen. However, only one isolate was positive on a repeat screen. To date, three VISA have been detected; two in 1999 and one in 2002.
Conclusion: The need to monitor the constant evolution of MRSA is evident. Further acquisition of resistance has been reported in 2002: high level resistance to vancomycin (*vanA* gene) was reported in the USA and resistance to Linezolid was reported in the UK. In addition clones with increased virulence are being detected: reports of 'Community-MRSA' which carry the Panton-Valentine Leukocidin toxin are increasing worldwide.

P1201 Evolution from 1991 to 2001 of oxacillin susceptibility testing methods for *Staphylococcus aureus* in Belgian laboratories

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Objective: To assess the laboratory methods used for detection of methicillin-resistant *S. aureus* (MRSA) in Belgian acute-care hospitals.
Methods: In 1991, 1995, 1997 and in 2001, a questionnaire about oxacillin susceptibility testing methods was mailed to all hospital labs ($n = 198$).
Results: The response rate was 59% ($n = 116$) in 1991, 43% ($n = 85$) in 1995, 58% ($n = 114$) in 1997 and 46% ($n = 92$) in 2001. The use of disk diffusion decreased from 91% in 1991 to 64% in 2001 while the use of oxacillin screen agar increased from 9 to 49%. Automated methods were used by 8% of the labs in 1991 compared with 24% in 2001. In 2001, the other methods were mecA gene detection by PCR ($n = 6$), MIC determination by *E*-test ($n = 3$) or latex agglutination of PBP2a ($n = 11$). The combination of methods increased strongly from 9 to 55% ($P < 0.001$). In 2001, 45, 9 and 1% of the labs used, respectively, two, three or four methods. In 2001, only 14 of 54 labs (24%) using disk diffusion and 20 of 47 labs (43%) using oxacillin agar screen strictly adhered to NCCLS guidelines. Discrepancies mainly concerned incubation time and temperatures. Of 41 labs using only one method in 2001, 20 (49%), 11 (27%) and 10 (24%) used, respectively, disk diffusion, oxacillin screen agar or automated methods. In the 42 labs using two methods in 2001, 12 different combinations have been observed. Combinations can be classified as follows:
- Disk diffusion with oxacillin screen agar (44%);
- Disk diffusion with automated or other method (32%);
- Oxacillin screen agar with automated or other method (18%);
- Association of two automated or other methods (6%).

The 10 labs using more than two techniques in 2001 have chosen disk diffusion and oxacillin-agar screen associated with automated or other methods.
Conclusions: The enhanced use of agar screen, automated methods and combination of different techniques enhanced the global accuracy for MRSA detection in Belgian laboratories. More standardization remains necessary and would allow a more accurate estimation of the overall sensitivity and specificity in Belgium based on test validity data from international scientific literature.

P1202 ESBL-producing nosocomial uropathogens

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Objectives: To isolate, identify and determine susceptibility of ESBL-positive uropathogens to antimicrobial agents.
Methods: Four thousand urine samples from hospitalized patients were examined in 2001. Seven hundred and thirty strains of Gram-negative rods were cultured. Identification and susceptibility were performed with automatic ATB Expression system (bioMérieux, France) using ID 32 E, ID 32 GN and ATB UR 5 strips. ESBL-producing strains were detected with double disc synergy test (DDST according to Jarlier et al. 1988) or a novel method of ESBL detection (DD, diagnostic disc) according to Appleton (1999). Two discs were applied in this test: CPD (cefpodoxime) and CD 01 (cefpodoxime/clavulanic acid) (Oxoid, UK).
Results: Six hundred and thirteen strains (84%) of enteric rods, 115 (15.7%) of nonfermenting rods and two (0.3%) of *Aeromonas* spp. were isolated. A hundred ESBL-producing strains (13.7% of all) were detected. Cultured ESBL strains included *E. coli* (30%), *K. pneumoniae* (18%), *E. Cloacae* (17%), *C. freundii* (15%), *K. oxytoca* (8%), other species of enteric rods (10%) and two strains of nonfermenting rods. Seventy-two percent of ESBL-positive uropathogens were susceptible to nitrofurantoin, 71% to fosfomycin and 67% to ciprofloxacin/norfloxacin. Ninety-nine percentage of strains were susceptible to carbapenems – imipenem and meropenem.
Conclusion: Nosocomial urinary tract infections were caused exclusively by Gram-negatives. ESBL-positive strains were detected most often among enteric rods. The most active drugs against examined uropathogens were carbapenems and nitrofurantoin.

P1203 Multi-resistant *Klebsiella pneumoniae* in pediatric intensive care unit

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Introduction: *Klebsiella* is one of the most frequent causative agents of hospital infection in children. Bacteria may originate from the environment and/or from the patients' mucosal surfaces. In the hospital the reservoirs for *Klebsiella* are the patients' gastrointestinal tract and the hands of hospital personnel. Hospital infections caused by multiresistant strains, especially extended-spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae* pose a problem in intensive care wards. The aim of our study was to estimate epidemiology of ESBL producing *Klebsiella* (ESBL-K) isolated from pediatric intensive care unit (PICU) patients, personnel and environment applying phenotypic and genotypic methods.
Material and methods: ESBL-K strains were isolated from children hospitalized during 28 months in Tartu PICU, from personnel and environment of the same ward. In order to detect the phenotype of ESBL-K strains the antibiotic susceptibility pattern and biotype (API E20) were tested. Genotype fingerprints were compared by pulsed-field gel electrophoresis (PFGE).
Results: In Tartu PICU 583 children were hospitalized in the period of January 2000 till April 2002. ESBL-K strains were isolated in 55 patients. No ESBL-K strains were isolated from the personnel ($n = 40$) and environment ($n = 192$). The 166 ESBL-K strains isolated from the patients were resistant to penicillins, cephalosporins and gentamicin but susceptible to carbapenems, tobramycin, amikacin and quinolones. Applying API E20 *K. pneumoniae* was detected in 64 strains out of 67 isolated from 26 patients. Using PFGE we found four different ESBL producing *K. pneumoniae* clones (A-D). The prevalent clone A was detected in 17 children out of 22.
Conclusions: During 28 months in 10% of patients hospitalized to PICU the ESBL-K was detected. The putative reason of circulating hospital infection was the most frequently isolated ESBL producing *K. pneumoniae* clone A. Unfortunately the transmission of the agent was not detected and needs

further investigation. We suggest the change of antibiotic use in the ward following the microbial susceptibility pattern.

P1204 Characterization of methicillin-resistant *Staphylococcus aureus* in a Portuguese hospital by multiple genotyping methods

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Objectives: To evaluate the clonal profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates recovered from a Portuguese hospital during two periods of surveillance (1992–1993 and 1996–2000).
Methods: A combination of molecular typing techniques: hybridization of *Cla*I digests with mecA and Tn554 probes and pulsed-field gel electrophoresis (PFGE) was used to identify the clonal types (mecA::Tn554::PFGE) of a total of 119 clinical MRSA isolates. Representative isolates of the different clonal types were further analyzed by spaA typing, multilocus sequence typing (MLST), and identification of the specific Staphylococcal Chromosomal Cassette (SCCmec) types.
Results: Two major MRSA clones were found: in 1992–1993, the internationally disseminated Iberian clone, I::E::A and related clonal types (ST247-SCCmecI), included the majority (77%) of the isolates. In 1996–2000 this clonal type decreased to 19% while a second internationally disseminated clone, XI::B::B (Brazilian) and related clonal types (ST239-SCCmecIIIA), emerged as dominant (69%). Nine sporadic/minor clonal types (representing 14% of the total of strains) were identified by mecA::Tn554::PFGE. However all but one of the selected representative isolates of these sporadic isolates revealed a spaA type, sequence type (ST), and SCCmec type identical to those of the major MRSA pandemic clones previously described namely the Iberian, Brazilian, and Pediatric (ST5-SCCmecIV). The unique and novel clone, ST79-IV, was a single locus variant of ST22 characteristic of the EMRSA-15 epidemic clone in UK hospitals and in other countries of Northern Europe.
Conclusions: Our findings document major shifts in the endemic MRSA background in this hospital since 1992 and reflects the Portuguese hospitals picture with the decline of the Iberian clone in the early 1990s in parallel with the increase of the Brazilian clone since 1995, suggesting a selective advantage of the Brazilian relatively to the Iberian clone. Interestingly the minor/sporadic clones in this hospital had been previously identified as epidemic in other countries including Portugal.

P1205 ESBL carriage among patients in a pediatrics public hospital in Gdansk, Poland

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Objective: The aim of this study was to analyze occurrence of ESBL producing Enterobacteriaceae in patients of outpatient clinics and wards of the Institute of Pediatrics (IP) in our hospital.
Methods and materials: We analyzed records of clinical materials received from IP patients in 2002. Strains were identified by classical method and GNI + VITEK cards (BioMérieux). Production of ESBL was detected by double disk method. ESBL colonization was determined by a positive stool/rectal swab culture or throat swab.
Results: In the studied period 3129 patients were hospitalized in IP. ESBL + isolates were recovered from 153 patients, 99 of them were inpatients and 54 outpatients. 59 inpatients were infected and 40 colonized, outpatients 41 and 13, respectively. Among outpatients only eight were earlier hospitalized in IP. Others previously attended different hospitals in our region. *Klebsiella pneumoniae* was most often isolated (249 isolates from 101 patients) then *E. coli* (102 isolates from 52 patients) and *K. oxytoca* (30 isolates from 16 patients), we infrequently cultured *E. cloacae* and *C. freundii* (three and two strains from four different patients, respectively). Isolates were most often recovered from urine (40%), stools and rectal swabs (31.8%), intravenous catheter tips (9.4%), respiratory tract (8.2%), soft tissues (2.4%), and blood (1.2%). 64.5% patients were admitted to IP units with ESBL. 35.5% patients acquired ESBLs during hospitalization. 28% patients received cephalosporins, 19% cotrimoxazole, 17% nitrofurantoin and 51% were not given any antibiotic *c.* 49% ESBL strains were detected in patients with urinary tract infections, 9% with diarrhea, and 4% with suspected sepsis. All strains were susceptible to carbapenems, about 90% to fluoroquinolones and about 50% urine isolates to nitrofurantoin.
Conclusions: Patients with ESBLs in outpatient clinics acquired them during hospitalization. Pediatric patients in our region should be screened for ESBL bacteria before discharge. High percentage of ESBL carriage among children complicates infection control and treatment of common urinary tract infection.

P1206 Abstract withdrawn

P1207 Antimicrobial resistance of nosocomial strains of *Enterococcus* spp. in Russia

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Objectives: The aim of this study was to determine the rates of antimicrobial resistance in nosocomial strains of *Enterococcus* spp. in Russia.
Methods: A total of 362 clinical strains of *Enterococcus* spp. isolated in 2000–2001 from patients hospitalized in 15 hospitals in different parts of Russia – four in Central region (Moscow, Ryazan, Smolensk), two in North-West region (St. Petersburg), two in South region (Krasnodar), two in Volga region (N. Novgorod, Kasan), two in Ural region (Ekaterinburg), three in Siberian Region (Krasnoyarsk, Novosibirsk, Tomsk), were included in the study. Antimicrobials tested included ampicillin (AMP), gentamicin (GEN), streptomycin (STR), vancomycin (VAN), teicoplanin (TEI), linezolid (LNZ), tetracycline (TET), chloramphenicol (CHL), ciprofloxacin (CIP), levofloxacin (LEV), moxifloxacin (MOX), quinupristin/dalfopristin (QD). Antimicrobial susceptibility testing was performed by agar dilution method. The susceptibility testing and interpretation of the results were performed according to the NCCLS guidelines.
Results: Among 362 strains 75.4% were *E. faecalis*, 16.3% – *E. faecium*, 8.3 – *Enterococcus* spp. (*E. durans*, *E. raffinosus*, *E. gallinarum*, *E. avium*). Results of susceptibility testing are presented in the Table 1.

Table 1

Antimicrobial	I + R (%)	MIC_{50}/MIC_{90}	MIC range
AMP	18.5	2/64	0.5–128
GEN	49.4	256/4096	8–4096
STR	54.1	4096/8912	32–8192
VAN	0	1/2	0.5–8
TEI	0	0.5/1	0.125–2
LNZ	0	2/2	1–2
QD	78.2	8/16	0.25–16
TET	68	64/128	0.5–128
CHL	55.8	16/64	2–128
CIP	72.7	2/32	0.5–128
LEV	17.4	2/8	0.5–64
MOX	N/A	0.5/8	0.125–32

Conclusions: (1) The most active antimicrobials to which no resistance has been found were linezolid and glycopeptides. (2) The most important problems determined were the high frequencies of resistance to ampicillin in *E. faecium* and to aminoglycosides in both *E. faecalis* and *E. faecium*.

P1208 Multiplex LightCycler real time PCR assay for the detection of vancomycin-resistant enterococci

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Objectives: In the United States, vancomycin resistant enterococci (VRE) have become common nosocomial pathogens. They account for 14% of enterococcal bacteremias in intensive care patients, exhibiting a mortality of 30–46%. Rapid identification of VRE is essential for the implementation of appropriate control measures to prevent the spread of VRE.
Methods: We developed a research kit for rapid real time PCR on the LightCycler Instrument (Roche) to identify the two main vancomycin resistance alleles in enterococci, vanA and vanB. The kit is based on multiplex

PCR employing specific primers and Hyb-probes for the detection of vanA, and vanB.
Results: Interestingly vanB specific PCR could detect an additional variant of the vanB allele called vanB2/3. All three alleles can be differentiated by melting point analysis. The whole procedure, including fully automated DNA preparation, PCR and data interpretation takes only 3 h. Sensitivity experiments revealed a detection limit of 5–10 genome equivalents.
Conclusion: Together, fast performance, sequence specificity and a very low detection limit makes the VRE kit a premium tool for detection of vancomycin-resistant Enterococci in research samples.

P1209 Nosocomial infections and misuse of antibiotics in a Golestan Province Hospital, north of Iran

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Objectives: Nosocomial infection surveillance is not common in the Iran but few hospitals use it. The incidence of nosocomial infection and prevalence of antibiotic misuse were studied in a 70-bed community hospital in Iran.
Methods: All pathogenic strains were tested for their susceptibility to antimicrobial agents using the standardized agar disc diffusion test. Mueller–Hinton agar and Mast antibiotic discs (Mast Diagnostics Ltd., Bootle, Merseyside, UK) were used. Different panels of antibiotics were employed for Gram-positive and Gram-negative organisms and also for urinary isolates. Susceptibility or resistance was determined on the basis of the size of the diameter of the zone of growth inhibition, according to the chart of interpretive standards.
Results: 45% of patients admitted developed nosocomial infection. The rates were highest for nursery (32%), intensive care (21%), gynecological (15.5%) and surgical (9.8%) patients. Urinary tract (39%), wound (24%) and blood (11.2%) infections accounted for more than 70% of the infections. *Staphylococcus aureus* (24.9%) and *Pseudomonas aeruginosa* (14.7%), caused more than 90% of the infections. The most frequent pathogens were *S. aureus*, *P. aeruginosa*, other Gram-negative aerobes and *Candida* spp. Some microorganisms such as *Klebsiella*, *E. coli* were resistance to five antibiotics. Coccobacill Gram-negative was resistance to three antibiotics. Some Gram-negative bacteria showed resistance to six antibiotics. The majority of the bacterial pathogens (75%) were multidrug resistant.
Conclusions: Overall, nosocomial infections were associated with an increased risk of antibiotics resistance. The results emphasize the need for effective measures to reduce both the high infection rates and widespread antibiotic misusage in the hospital. Such measures should include institution of an effective infection control committee and a hospital antibiotic policy. It is also necessary to introduce urgent measures for control of antibiotic misuse and restriction of the spread of *P. aeruginosa* and *S. aureus*.

P1210 Prevalence and molecular epidemiology of PER-1 beta-lactamase in Gram-negative bacteria

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Objectives: To investigate the prevalence of PER-1 beta-lactamase among Gram-negative bacteria (GNB) isolated at a tertiary care hospital in Izmir, Turkey and to analyze the clonal relationship of PER-1 producers.
Methods: PER-1 beta-lactamase production was sought in 155 ceftazidime resistant GNB (53 *Pseudomonas aeruginosa*, 52 *Acinetobacter baumannii*, 25 *Escherichia coli*, 25 *Klebsiella pneumoniae*) isolated between January 1999 and September 2002. blaPER-1 presence was determined by PCR and clonal relationship of PER-1 producers was analyzed by ERIC PCR.
Results: Fifteen (28.3%) *P. aeruginosa* and 20 (38.5%) *A. baumannii* isolates were found to possess blaPER-1. On the other hand, all Enterobacteriaceae except one *K. pneumoniae* (4%) were blaPER-1 negative. Two main clusters were detected both among *P. aeruginosa* and *A. baumannii* isolates. Although most of the strains with identical ERIC PCR patterns were isolated from the intensive care units (ICUs), *A. baumannii* that exhibited the same pattern were isolated from different hospital wards.
Conclusions: PER-1 enzyme producers have been well established in our hospital especially in the ICUs. Our results suggest that dissemination of endemic clones was responsible for the high prevalence of PER-1 production among nonfermentative bacteria.

P1211 Nosocomial infections caused by *Pseudomonas aeruginosa* with acquired blaVIM metallo-beta-lactamase genes in a hospital in Northern Italy

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Objectives: To investigate the diffusion of strains producing metallo-beta-lactamases (MBLs) among carbapenem-resistant *Pseudomonas aeruginosa* isolated from nosocomial infections in a hospital of northern Italy where MBL producers were first detected in 1998.
Methods: Seventy-six nonreplicate carbapenem-resistant *P. aeruginosa* isolated at the University Hospital of Pavia since 1998 were included in the study. Susceptibility testing was carried out by a broth microdilution method. The EPI microdilution test, in which MICs of imipenem (IMI) are determined with and without the presence of a mixture of chelators, was used for screening of MBL production. Carbapenemase activity was estimated by spectrophotometry. The presence of blaVIM- and blaIMP-type genes was investigated by multiplex PCR, and the nature of the determinant was identified by the restriction profile of the amplification products. Pulsed-field gel electrophoresis (PFGE) profiles of genomic DNA digested with *Spe*I were analyzed using the Bio-Rad Gene Path Procedure.
Results: Six of the 76 (7.8%) *P. aeruginosa* isolates exhibited a positive result in the EPI test. Photometric assays showed that crude beta-lactamase extracts exhibited a comparable carbapenemase activity. Molecular analysis revealed the presence of blaVIM determinants (5 blaVIM-1 and 1 blaVIM-2) in the six isolates. All but one exhibited IMI MICs > 64 mg/L. VIM producers were isolated during the period 1998–2002, from four different wards, and from different types of nosocomial infections (urinary tract, decubitus ulcer, respiratory tract). Only in some cases they were epidemiologically and clonally related.
Conclusion: These results indicated a low but persistent prevalence of nosocomial infections caused by *P. aeruginosa* producing VIM-type MBLs at this hospital. These strains exhibit a low ability to cause nosocomial outbreaks. The EPI test was successful for rapid detection of MBL producers.

P1212 Epidemiology and frequency of antimicrobial resistances among pathogens causing surgical site infections: report from the SENTRY Antimicrobial Surveillance Program in the United States, Canada and Europe

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Objective: Surgical site infections (SSIs) cause considerable morbidity, increase hospital length of stay, add to healthcare costs, and are largely preventable via good infection control practice and appropriate use of prophylactic antimicrobials. We addressed SSI in the SENTRY Program in 1999.
Methods: We collected between July and December over 600 isolates (20 consecutive isolates/center) from surgical patients hospitalized in 40 medical centers (US, 26; Canada, five; and EU, nine [five nations]). At the monitoring center (Iowa), isolates were further processed using standardized testing and NCCLS susceptibility criteria (25 antimicrobials). Molecular epidemiologic typing of selected strains was also performed.
Results: Overall, 512 isolates were evaluable as causing SSI and patient demographics included: mean age, 56 years (range 1 month−95 years); males 55.8%; median duration of hospital stay was 11 days; and average onset of infection at 7 days (82% within 30 days). One-third of the specimens (commonly swabs 60.3%, aspirate 18.9%, tissue 11.3%) were obtained from patients located in the surgical ICU and operating rooms. Body sites included abdomen (34.3%), limb (24.4%), chest (17.2%) and cranio-spinal areas (8.9%). Procedures included coronary artery bypass (7.7%), appendectomy (5.3%), and mastectomy (3.5%). Over 30 preoperative antimicrobials were utilized with ceftazidime in 25% of cases and one-half of the patients received more than one drug class. Rank order of pathogen occurrence was: *S. aureus* (SA; 34.5%) > *P. aeruginosa* (PSA; 13.5%) > *E. coli* (EC; 12.7%) > enterococcus

(E) > *Enterobacter* spp. > *Klebsiella* spp. Regional variations were noted in pathogen prevalence (example *P. aeruginosa* was the most often isolated species in EU). Polymicrobial infection was observed in less than 5% of cases. Overall, methicillin resistance among SA was highest in EU (43.8%), and vancomycin-resistant-E (14.3%) in the US. ESBL phenotype rates among *Klebsiella* spp. (8.4%) and EC (7.1%) were modest, but least in the US (0–3.3%). In the US, imipenem was most active agent against PSA (97.3%) > piperacillin/tazobactam > gentamicin > cefepime > ceftazidime.
Conclusion: Resistance rates among nosocomial strains causing SSI vary widely in different geographical regions. Global and local surveillance must continue as a tool to limit the emergence and better define prophylactic antimicrobial choices appropriate for surgical procedures.

Pharmacokinetics

P1213 Effect of rifampicin on the pharmacokinetics of dapsone and its metabolite, monoacetyldapsone in HIV-infected patients

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The dual pulmonary infections with both *Mycobacterium tuberculosis* and *Pneumocystis carinii* in AIDS patients have been found more common than previously report. Dapsone has been increasing use as an alternative agent for PCP prophylaxis and the CYP3A4 system is responsible for its metabolism. Rifampicin is still one of the most valuable drugs for the standard treatment of tuberculosis and known to be a potent inducer of hepatic cytochrome P450 enzymes. The objective of this study was to investigate the effects of rifampicin on the pharmacokinetics of dapsone and monoacetyldapsone. Our study was conducted in 12 HIV-infected patients. All patients received 100 mg of oral dapsone once daily in the morning from day 1–7 and day 11–17 and 600 mg of oral rifampicin once daily in the morning from day 11–20. Dapsone and monoacetyldapsone pharmacokinetic studies were carried out on day 7–10 and day 17–20 (96 h). The results showed that dapsone and monoacetyldapsone concentrations were lower when dapsone was administered with rifampicin than when it was administered alone. The mean AUC0–24, C_{max} and C_{min} of dapsone were decreased by 76.08, 48.21 and 88.23%, respectively, and the mean AUC0–24 and C_{max} of monoacetyldapsone were decreased by 83 and 61.95%, respectively, and the mean C_{min} of monoacetyldapsone was undetectable. Thus, we conclude that rifampicin has an inducing effect on the metabolism of dapsone and its metabolite, monoacetyldapsone.

P1214 Pharmacokinetic disposition and serum bactericidal activity following IV infusion of single and multiple ascending doses of TD-6424 in healthy male subjects

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Introduction: TD-6424 (TD) is a new antibiotic that exerts rapid, concentration-dependent bactericidal activity against clinically significant Gram-positive bacteria, including methicillin-resistant *S. aureus* (MRSA) and penicillin-resistant pneumococci (PRSP). TD inhibits bacterial lipid synthesis in addition to peptidoglycan synthesis.
Objectives: To determine the pharmacokinetics (PK) of TD in healthy males and quantify serum bactericidal activity (SBA) against MRSA and PRSP.
Methods: Fifty-four subjects received single [SD] ($N = 27$) or multiple [MD] doses ($N = 27$) of TD or placebo in this double blind, randomized, placebo-controlled study. At each SD level, six subjects received TD and two received placebo. At each MD level, seven subjects received placebo and seven received TD. SD ranged from 0.25 to 15 mg/kg, and MD ranged from 7.5 to 15 mg/kg given q24hr by 30 min IV infusion for 7 days. Blood and urine samples were taken at regular intervals to evaluate PK and SBA (at peak and trough). Plasma samples were analyzed with a validated LC/MS assay, and PK parameters were derived using nonlinear methods. SBA titers were assessed vs. one strain each of MRSA (ATCC 33591) and PRSP (clinical isolate), using standard NCCLS methods, including addition of human serum.
Results: Following SD, TD exhibited approximately linear PK. Following MD, AUC, C_{max}, and $T_{½}$ were not significantly different from those observed at Day 1. Little accumulation was observed and only C_{min} was slightly increased. Steady state was achieved by Day 3. Following Day 7 dosing, the PK results for TD at the dose range proposed for clinical use (7.5–15 mg/kg) were as follows: peak plasma concentrations and AUCss for 7.5, 12.5 and 15 mg/kg doses were 97, 151 and 203 mg/L, and 700, 1033, and 1165 mg h/L, respectively. $T^{½}$ and Vss were approximately 9 h and 0.1 L/kg, respectively. Median peak/trough SBA titers for the three doses vs. MRSA were $256/16 \geq 512/24$, and $\geq 512/32$, respectively; and vs. PRSP were $\geq 512/128$, $\geq 512/256$, and $\geq 512/\geq 512$, respectively.
Conclusions: The PK of TD following SD in healthy volunteers appeared to be largely independent of dose. C_{max} and AUC values of TD demonstrate approximate dose proportionality. Following MD, the PK of TD was similar to that following SD, and no significant accumulation was observed. Excellent SBA persisting for 24 h was observed against the strains of PRSP and MRSA. These results are suggestive of potential efficacy in the treatment of Gram-positive infections.

P1215 Application of microdialysis to corticocancellous bone tissue for the evaluation of gentamicin: an experimental study

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Objectives: Most antimicrobial agents exert their effect inside the interstitial space, which is the site of many infections. For the evaluation of antimicrobial agents in bone tissue, mainly studies of bone specimens have been performed. Microdialysis is a new technique that allows dynamic and continuous in vivo sampling. The principle is to introduce a membrane into the tissue and perfuse it with a liquid that equilibrates with the fluid outside of the membrane by diffusion, thus enabling dynamic measurements to be made. The aim of this investigation was to introduce microdialysis to corticocancellous bone tissue for the evaluation of gentamicin and compare it to values obtained from bone specimens.
Methods: Eight pigs (46.4 ± 0.7 kg, creatinin 144.3 ± 7.9 mM) were included into the study and underwent surgery in general anesthesia. All animals received an intravenous injection of 240 mg gentamicin. Concentrations of gentamicin were measured in serum and by the technique of microdialysis on an Abbott Drug Analyzer. A direct agar diffusion technique was used to evaluate bone specimen gentamicin using *S. epiderminis* ATCC 12228. Data presented are means ± SEM. A *P*-value below 0.05 was considered significant. The pharmacokinetic measure used was the area under the curve from 0 to 6 h (AUC 6 h). All surgical procedures were performed under the approval and guidelines of the Danish Ministry of Justice, Animal Experimentation Inspectorate.
Results: The peak concentrations of the two microdialysates and bone specimens were 6.73 ± 0.8 mg/L, 6.52 ± 1.09 mg/L and 5.49 ± 1.02 mg/L. Similar the area under the curve from 0 to 6 h (AUC6 h) were 1569 ± 198 mg/min/L, 1721 ± 248 mg/min/L and 1390 ± 121 mg/min/L (ANOVA, $P = 0.5$). Serum gentamicin peaked at 33.31 ± 2.66 mg/L. Reproducibility of the measurement from the microdialysates was evaluated from the mean AUC6 h/catheter no. 1/AUC6 h/catheter no. 2 ratio. This ratio was 1.02 ± 0.17.
Conclusion: The evaluation of bone specimens is a difficult task and frequent sampling on the same individual is often not possible due to ethical considerations. It seems that microdialysis is a suitable, relative noninvasive and reproducible technique for dynamic and quantitative measurements of gentamicin in corticocancellous bone tissue.

P1216 Urinary concentration-vs.-time profile of levofloxacin 500 mg bid in ICU patients treated for ventilator associated pneumonia

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Objectives: The aim of our study was to assess the urinary pharmacokinetics of LFX by collecting urine samples at appropriate intervals in ICU patients treated with LFX 500 mg b.i.d. i.v. because of a ventilator associated pneumonia (VAP).
Methods: In steady-state condition, urine samples were collected during a dosing interval (at 0–2, 2–4, 4–8 and 8–12 h), and urinary concentrations of LFX were assayed by HPLC. The study involved 12 patients (6M, 6F; age, 57 ± 19 years; weight, 71 ± 11 kg) presenting with a normal renal function (estimated creatinine clearance, 1.87 ± 0.66 mL/min/kg; diuresis, 1825.4 ± 575.3 mL/24 h).
Results: Mean (± standard deviation) of LFX urinary concentrations were 358.2 ± 169.9 mg/L, 392.7 ± 154.7 mg/L, 241.1 ± 85.9 mg/L and 139.9 ± 71.9 mg/L at 0–2, 2–4, 4–8 and 8–12 h, respectively. Fractional urinary excretions of LFX were $20.0 \pm 8.9\%$, $17.0 \pm 3.0\%$, $26.1 \pm 5.3\%$, $15.5 \pm 3.6\%$ at 0–2, 2–4, 4–8 and 8–12 h, respectively, with a total cumulative excretion of $78.7 \pm 12.9\%$ in the overall dosing interval.
Conclusions: Our findings show that LFX 500 mg bid iv enables to maintain high urinary concentrations during the overall dosing interval in patients with normal renal function, and confirm that LFX is excreted mainly as unchanged drug by the renal route. Mean urinary concentrations were 50–175-fold higher than the breakpoint for sensitive bacteria during the overall dosing interval. Considering that a peak level (C_{max}) to minimum inhibitory concentration (MIC) ratio higher than 12.2 (C_{max}/MIC > 12.2) was shown to be one of the main pharmacodynamic determinant for the concentration-dependent bactericidal activity of LFX, we can conclude that the urinary pharmacokinetics of LFX enables an optimal exposure for the treatment of UTIs not only against sensitive microrganisms, but probably also whenever microrganisms usually considered as moderate sensitive or resistant to LFX if localized in other infection sites may be involved.

P1217 Evaluation of the effect of age, weight, race and gender on the plasma concentrations of posaconazole in HIV-infected patients with oropharyngeal or esophageal candidiasis

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Background: Posaconazole (POS) is a triazole antifungal in clinical development for the treatment of refractory oropharyngeal (OPC) and esophageal candidiasis (EC) and invasive fungal infections. The pharmacokinetics (PK)of some antifungal agents are affected by covariates such as age, gender, and race. This analysis was performed to assess whether individual patient plasma levels at steady state (Css avg) were altered in patients who varied with respect to age, weight, race, and gender.
Methods: A total of 199 subjects were enrolled in an open-label, multicenter, noncomparative efficacy and safety study of POS for the treatment of azole-refractory OPC or EC. POS oral suspension (400 mg/10 mL) b.i.d. with food was used for 4 weeks as acute treatment. Of these treated subjects, 46 were randomly chosen from the data set for examination of serum POS concentrations. A total of 70 samples of venous blood (one or two per subject) were collected per protocol prior to dosing on Days 8 and 28. POS levels were analyzed using liquid chromatography with tandem mass spectrometric detection. Graphical correlations with regression analyses were performed to determine if a trend between the various covariates and the plasma concentrations was observed.
Results: A mean Css avg of 577 ng/mL was observed across the entire population, with a mean time of 11.2 h between dosing and sampling. There were no correlations observed between the individual patient POS Css avg values and the four demographic factors evaluated. Mean Css avg values were not associated with differences in age (25–54 years) or weight (37–94 kg) between patients; linear regressions yielded R2 values of 0.012 and 0.038, respectively. The 90% confidence intervals for the mean Css avg values overlapped in samples taken from whites ($n = 43$), blacks ($n = 23$), and Hispanics ($n = 4$), indicating that there was no effect of race on the plasma concentrations of POS. Although the number of samples available from women ($n = 9$) compared with men ($n = 61$) was small, mean Css avg values between the genders were statistically equivalent.
Conclusions: Age, weight, race, and gender had no effect on POS plasma concentrations at steady state in HIV-infected patients with OPC or EC. Correlation of PK parameters and clinical and mycologic responses are ongoing as part of the larger data analysis in this study. Larger studies and a population analysis assessing the role of these covariates on the PK of POS are currently ongoing.

P1218 Relative oral bioavailability of three formulations of posaconazole in healthy volunteers: basis for clinical development of the suspension

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Objectives: Posaconazole (POS) is a new triazole antifungal that has activity against a wide variety of pathogens including *Aspergillus* spp. and *Zygomycetes*. Two Phase I pharmacokinetic (PK) studies were conducted to determine the relative oral bioavailability of a suspension, capsule, and tablet formulation of POS under fed conditions in healthy male volunteers.
Methods: Both studies employed randomized, open-label, single-dose designs. In Study 1, 20 subjects received either a single 200-mg dose of the POS oral suspension (5 mL) or POS tablets (2×100 mg) after a high-fat breakfast (>50% of calories from fat) in a crossover manner. In Study 2, 24 subjects received either POS tablets or capsules (both 2×100 mg) after a high-fat breakfast. There was a 1-week washout between treatment periods in both studies. Venous blood was collected predose and up to 72 h postdose for determination of POS concentrations. PK parameters were calculated using model-independent methods.
Results: The balanced mean POS PK parameters are shown in Table 1.

Table 1

Parameter	$AUC_{(0-inf)}$ (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)
Study 1				
Suspension ($n = 20$)	15059*,a	512*,b	4.8	23.0*
Tablets ($n = 20$)	10304*	413	5.5	21.0*
Study 2				
Capsules ($n = 21$)	14293c	531d	5.1	26.1
Tablets ($n = 21$)	12505	432	6.2	25.5

*n=15.
[a]$P = 0.001$ vs. tablets; [b]$P = 0.041$ vs. tablets; [c]$P = 0.089$ vs. tablets; [d]$P = 0.103$ vs. tablets.

The POS AUC(0–inf) values were ~5 and 46% greater for the oral suspension vs. the capsule and tablet formulations, respectively. The increase in exposure (AUC(0–inf) and C_{max}) of the suspension relative to the tablet was statistically significant ($P \leq 0.041$). The AUC and C_{max} values of the capsule and tablet did not differ significantly ($P \geq 0.089$). In these two independent studies where POS was administered as a tablet, similar PK parameters of POS were observed between studies, indicating there were no significant interstudy differences in the PK of POS.
Conclusions: The POS suspension had greater relative oral bioavailability than the tablet and capsule formulations. Based on these results, the clinical development of POS has progressed using the suspension in order to deliver optimal drug exposure in severely ill patients. In addition, POS suspension may allow easier administration and improved tolerability in patients with mucositis or receiving medications via a feeding tube. The suspension may also provide an additional topical antifungal effect in patients with oropharyngeal candidiasis.

P1219 The effects of food and fat content on the relative oral bioavailability of two formulations of posaconazole

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Objectives: Posaconazole (POS) is a potent new triazole antifungal in Phase III clinical development for the treatment and prophylaxis of serious fungal

infections. Two studies were conducted to determine the role of food and fat content on the systemic exposure of the suspension and tablet formulations of POS.
Methods: Both studies were randomized, single-dose, open-label and cross-over in design and were conducted in healthy male volunteers. In Study 1, 20 subjects received a single 200-mg dose of POS oral suspension (5 mL) after a 10-h fast, with a nonfat breakfast, or a high-fat breakfast (>50% of calories from fat); or POS tablets (2 × 100 mg) with a high-fat breakfast. In Study 2, 12 subjects received a single POS tablet (1 × 200 mg) after a 10-h fast or with a high-fat breakfast. In both studies, there was a 1-week washout between treatments. Venous blood was collected predose and up to 72 h postdose for the determination of POS concentrations. Pharmacokinetic (PK) parameters were calculated using model-independent methods.
Results: Balanced mean PK parameters are shown in Table 1.

Table 1

Parameter	C_{max} (ng/mL)	T_{max} (h)	$AUC_{(tf)}$ (ng h/mL)	$t_{1/2}$* (h)	CL/F* (mL/min)	Vd/F* (L)
Suspension (Study 1)						
Fasted ($n=20$)	132	5.0	3553	23.5	858	1743
Nonfat meal ($n=20$)	378	4.1	9511	22.2	350	676
High-fat meal ($n=20$)	512	4.8	13885	23.0	234	454
Tablets (Study 2)						
Fasted ($n=12$)	92.5	9.0	2718	19.0	1062	1709
High-fat meal ($n=12$)	366	7.6	10220	18.3	323	526
High-fat meal ($n=20$; Study 1)	413	5.5	10304	21.0	362	648

*$n=15$ (Study 1); $n=7$ (Study 2).

Systemic exposure (AUC(tf)) of POS was ~4-fold greater after a high-fat meal than after a 10-h fast ($P \leq 0.001$) and was independent of formulation administered. The intake of food had a greater influence on the relative oral bioavailability of POS than the fat content of the meal. Compared with the fasted state, administration of a nonfat meal resulted in a 167% increase in POS systemic exposure (9511 ng h/mL vs. 3553 ng h/mL), while the addition of fat calories only increased exposure by an additional 46% (13 885 ng h/mL vs. 9511 ng h/mL). In these two studies in which POS was administered as a tablet with a high-fat meal, similar PK parameters of POS were observed between studies, indicating there were no significant interstudy differences in the PK of POS.
Conclusions: Food increased the relative oral bioavailability of POS; this increase was independent of the formulation administered. In addition, food containing fat calories further enhanced the relative oral bioavailability of POS.

P1220 Therapeutic drug monitoring of cotrimoxazole: a 9-year review of levels from a UK clinical antibiotic assay service

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Objectives: Co-trimoxazole (SMZ–TMP) is a mixture of trimethoprim (TMP) and sulfamethoxazole (SMZ) in the proportions 1:5 TMP:SMZ and is the drug of choice in *Pneumocystis carnii pneumonia* (PCP) where high dose treatment is given (20 mg/kg/day). Therapeutic monitoring is useful in treatment for PCP, particularly in patients with renal failure in order to achieve both therapeutic serum levels and avoid risk of toxicity. Normal serum levels are usually in the ratio of 20:1 (SMZ:TMP), with guide-line levels of pre dose SMZ < 100 mg/L; TMP 5–7 mg/L but < 20 mg/L and post dose SMZ 120–150 but <200 mg/L; TMP 5–10 but <20 mg/L. Achievement of optimum levels of TMP can result in toxic levels of SMZ and conversely impaired renal function can reduce the renal excretion of TMP resulting in accumulation whilst the SMZ is still metabolized. We reviewed 9 years of SMZ–TMP levels from our antibiotic assay service and present our findings.
Methods: SMZ and TMP were assayed simultaneously by HPLC. Data was collected retrospectively from the hospital Laboratory Information Management System.
Results: From January1994–October 2002 there were 593 sets of patient sera for SMZ–TMP assay; a total of 279 individual patients from 93 different requester locations. Of the 303 pre dose levels requested 47.5% were above the SMZ guideline level of 100 mg/L and of the 310 post doses assayed 21.9% were above the potentially toxic SMZ level of 200 mg/L. 6.3% of pre dose TMP samples and 13.9% of post dose TMP samples were above the toxic concentration of 20 mg/L. 16.8% of TMP post dose levels were below the optimum therapeutic level of 5 mg/L. Of the paired pre and post dose SMZ–TMP serum samples ($n=274$), 36.9% of TMP post dose levels were in the normal range when SMZ pre or post levels were potentially toxic with 16.1% having an inadequate TMP post dose level when the SMZ levels were within normal range. In only 5.1% were the concentrations of both the agents in the optimum range.
Conclusions: A significant proportion of samples assayed for SMZ–TMP are at potentially toxic levels as a result of elevated SMZ levels. Coupled with the knowledge that greater than 1 in 6 post dose TMP levels are potentially therapeutically inadequate (<5 mg/L), dose adjustment involving a reduction in the dose of SMZ–TMP with an additional TMP only supplement and vigilant monitoring of serum levels may improve achievement of optimum therapeutic levels with a reduced risk of SMZ toxicity problems.

P1221 The pharmacokinetics of 800 mg teicoplanin (T) given once every 48 h

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Objectives: T is extensively used for outpatient intravenous therapy in the UK and Europe as it can be given as a once-daily dose of 400 mg. The pharmacokinetics of T are best described using a three-compartmental model which indicates a terminal half-life of up to 182 h. Alternate day dosing has been used to treat patients with home i.v. therapy. The pharmacokinetics of 800 mg T over 48 h were therefore determined in inpatients with orthopedic infection.
Methods: Seven consenting inpatients (six male, one female) with normal renal function were studied; mean age 67 years (range 35–73), mean weight 93 kg (range 65–114). 800 mg T was administered in 100 mL saline as a 10–20 min infusion. Blood was collected at times; 0.08, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48 h after start of infusion. T serum concentrations were assayed by Polarization Fluorescent Immunoassay (FLX Abbott). Data were analyzed using WinNonlin.
Results: Best fit was obtained using a three-compartment model. Mean concentration observed 20–30 min after the start of infusion was 132.0 mg/L (range 107.4–148.8) and mean 48 h concentration was 3.6 mg/L (range 2.5–5.0 mg/L). 12 h after the end of infusion the mean serum concentration was 8.8 mg/L (95% CI 7.1–10.5). Mean AUMC was 17 127 mg h/L (95% CI 10 957–23 297). Half-lives of the alpha and beta phases were 00.17 h (95% CI 0.13–0.21) and 01.68 h (95% CI 1.24–2.12), respectively, which are comparable to those reported following single doses of 200 and 400 mg. The mean terminal half life, 23.8 h (95% CI 19.4–28.14), VdistributionSS 0.37 L/kg (95% CI 30.16–36.12) are lower than previously reported, this is consistent with our very short sampling period (48 h).
Conclusion: T 800 mg pharmacokinetics when administered 48 h is best described using three-compartment modeling and the pharmacokinetic parameters are in keeping with those for 400 mg 24 h allowing for the short sampling period. However, without prior loading doses 800 mg 48 h is insufficient to reliably achieve trough concentrations of >10 mg/L.

P1222 Anidulafungin dosage adjustments are not required for patients with hepatic and/or renal impairment

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Objectives: Anidulafungin, an echinocandin, is being developed for the treatment of serious fungal infections. Anidulafungin is not metabolized by hepatocytes or CYP450 enzymes, but is nonenzymatically degraded. The drug and degradation products are excreted in bile/feces and not in urine. In anticipation of treating patients with hepatic impairment (HI) or renal impairment (RI), studies were conducted to evaluate whether dosage adjustments are necessary.
Methods: Two studies were conducted to examine the safety and pharmacokinetics (PK) of anidulafungin in subjects with either HI or RI vs. subjects with normal function. PK were obtained from six subjects/group: normal hepatic function and mild, moderate, or severe HI (Child–Pugh classes A, B, and C). RI was defined by creatinine clearance (mL/min): mild = 51–79, moderate = 31–50, and severe <30. PK were obtained from 8 subjects/group for normal renal function and mild RI and 6 subjects/group for moderate, severe, and end-stage (on dialysis) RI. Subjects were given a single 50 mg i.v. dose of anidulafungin,

and plasma samples for PK analysis collected for 6 days postdose. Safety was assessed by physical exam and adverse event/laboratory monitoring.
Results: PK in subjects with normal hepatic function and those with mild/moderate HI were similar. In subjects with severe HI, decreases were observed in the maximum concentrations (C_{max}) and area under the concentration–time curve (AUC) and increases in drug clearance and volume of distribution; these are changes that are likely secondary to ascites and edema. PK in subjects with normal renal function and those with RI were similar. Additionally, there were no measurable amounts of anidulafungin found in dialysate. The drug was well-tolerated. No dose-limiting toxicities or serious adverse events occurred. The mean PK parameters are shown in Table 1.

Table 1

	C_{max} (mg/L)	AUC (mg h/L)
Hepatic function		
Normal	2.9	70.0
Mild HI	2.2	56.0
Moderate HI	2.3	68.6
Severe HI	1.8	46.6
Rural function		
Normal	2.1	51.1
Mild RI	2.2	52.5
Moderate RI	2.7	61.4
Severe RI	2.3	54.2
End-stage	2.2	52.7

Conclusions: The PK of anidulafungin were not affected in any clinically relevant way by either HI or RI. The drug can be administered to patients with HI or RI, without dosing adjustments, or without regard to the timing of hemodialysis.

P1223 Anidulafungin biotransformation in humans is by degradation not metabolism

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Objectives: Anidulafungin (ANID), an echinocandin, is being developed for the treatment of serious fungal infections. Studies were conducted to support clinical development by studying the pharmacokinetics (PK) of the drug in animals and humans and to determine the extent and mechanism of metabolism and drug elimination.
Methods: PK studies were performed in rats receiving an i.v. infusion of 5 mg/kg of ANID. A mass-balance study, using [C-14]-ANID was performed in rats and in human volunteers (100 mg/100 μCi). In vitro studies were performed with incubations in buffer, plasma, and media containing microsomes or isolated hepatocytes.
Results: Following administration of [C-14]-ANID in rats and humans, drug-derived radioactivity persists longer than ANID. In humans, the half-lives of ANID and drug-derived radioactivity are 28 and 119 h, respectively. These results would suggest extensive metabolism of the drug, however, in vitro studies show the drug is not metabolized when incubated in primary human hepatocytes, nor is it a substrate for CYP450 enzymes. ANID was found to be degraded in human plasma, with a kinetic half-life (~1 day) that is similar in buffer (pH 7.4, 37 °C), in vitro plasma (37 °C), animal PK studies, and human PK studies. The fact that the in vitro degradation rate in buffer is similar to the degradation rate in plasma indicates ANID in vivo degradation is non-enzymatic. The primary degradation product is a linear peptide that is further enzymatically degraded to tertiary products. Drug and labeled degradation products are not found in urine. The majority of drug is degraded, with tertiary degradation products eliminated in the feces via bile. A small amount of intact drug (<10%) is also eliminated in feces. ANID has a volume of distribution of approximately whole body fluid; the volume of distribution in the rat and human is 1.7 L/kg and 37 L, respectively.
Conclusions: ANID is eliminated in the body through biotransformation by nonenzymatic degradation that is time, pH, and temperature-dependent. The lack of hepatic metabolism and urinary excretion may explain previously reported studies showing ANID concentrations are not increased due to hepatic or renal impairment, unlike another echinocandin. Additionally, these results indicate that ANID has a low potential for drug interactions.

P1224 Dalbavancin dosage adjustments not required for patients with mild renal impairment

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Objectives: Dalbavancin is a novel semisynthetic glycopeptide in phase 3 clinical development, that has activity against Gram(+) organisms, including resistant strains. Weekly doses have been shown to be effective in deep skin and soft tissue infections. The drug's half-life supports once-weekly dosing, and it is eliminated through both renal and nonrenal routes. Because patients with renal impairment (RI) are likely to receive dalbavancin, clinical studies were done to determine the need for dose adjustments in subjects with mild RI.
Methods: Subjects enrolled in the clinical studies received dalbavancin as a single i.v. infusion (70 or 1000 mg). All subjects had mild RI as defined by creatinine clearance (CrCL) of 50–80 mL/min. Plasma samples were collected through at least 14 days after the dose. Dalbavancin was assayed using validated LC-MS/MS methods. Pharmacokinetic (PK) data were analyzed using noncompartmental methods. PK of subjects with mild RI were compared with normal subjects (CrCL > 80 mL/min) from a previous study.
Results: Seven subjects with mild RI were enrolled and received one dose of either 70 or 1000 mg of dalbavancin. Six subjects with normal renal function were enrolled in a separate study and were used for comparison. Table 1 summarizes the mean (SD) of PK parameters from these studies. Dalbavancin PK parameters, including maximum concentration (C_{max}) and the area under the concentration–time curve through 14 days (AUC0–14), and concentration–time profiles were similar between subjects with mild RI receiving either the 70 or 1000 mg dose and subjects with normal renal function Table 1.

Table 1

	N	C_{max} (mg/L)	C_{max}/Dose	AUC0–14 (mg h/L)	AUC0–14/ Dose
Mild renal impairment					
70 mg	3	22 (6)	0.32	1299 (312)	18.6
1000 mg	4	276 (107)	0.28	15910 (996)	15.9
Normal renal function					
1000 mg	6	301 (65)	0.30	16772 (3193)	16.8

Conclusion: Dalbavanicin does not require a dosage adjustment for patients with mild RI. These results are consistent with previous clinical and non-clinical PK studies showing that dalbavancin, unlike currently marketed glycopeptides, has dual (both renal and nonrenal) routes of elimination. Studies are ongoing to determine if dosage adjustments of dalbavancin will be required for subjects with moderate, severe, or end-stage (on dialysis) RI.

P1225 Attributes of dalbavancin: well distributed, weekly dosing, and completely eliminated

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Objectives: Dalbavancin is a novel semisynthetic glycopeptide in phase 3 clinical development, that has activity against Gram(+) organisms, including resistant strains. Weekly doses have been shown to be effective in deep skin and soft tissue infections. Studies were performed to support the clinical development of dalbavancin by investigating drug distribution and excretion.
Methods: Two studies were performed in rats administered a single i.v. infusion of 20 mg/kg [H-3]-dalbavancin. Excreta and more than 40 different tissues were collected through 70 days, and the distribution and kinetics of drug-derived radioactivity were examined. Results from animal studies were compared with humans using pharmacokinetic data from volunteers that were administered an i.v. infusion of 1000 mg dalbavancin.
Results: In rats, approximately two-thirds of the excreted drug was found in urine, and one-third was observed in feces. Approximately 25% of the dose is eliminated after 1 day and almost 60% after 1 week. This is consistent with a plasma $t_{1/2}$ of approximately 1 week. The majority of fecal drug elimination is via excretion in bile. Blood to plasma ratio remained relatively constant over

time and <1. Concentrations and half-lives of radioactivity in tissues were comparable to that observed in plasma. Concentrations in skin were comparable to or higher than values observed in plasma, while concentrations remained relatively low in the CNS in this healthy animal model. At 12 h postdose, all tissues had quantifiable concentrations of radioactivity with peak concentrations attained within 24 h after the dose. Less than 5% of the dose was found in any one tissue 5 days following the dose. By 10 weeks after the dose, less than 5% of the dose remained in the carcass. The entire dose was accounted for through-out the study. Elimination of dalbavancin in humans was similar to rat; the plasma $t_{1/2}$ was 9–12 days and approximately 40% of intact drug was excreted in urine.
Conclusions: These results support the use of dalbavancin as a once-weekly regimen, show the drug to be well distributed including good penetration into skin, and have both renal and nonrenal routes of elimination. Dual routes of elimination may be a desirable attribute for patients with renal or hepatic impairment, and other special populations. The drug does not accumulate into any one tissue, compartment, or organ.

P1226 Modulation of the cellular accumulation of garenoxacin (BMS284756) and levofloxacin in J774 macrophages

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Objectives: Ciprofloxacin accumulates in J774 macrophages and is subject to active efflux through multidrug resistance related protein (MRP). Inhibition of this transporter increases, indeed, its accumulation to 300% of control values (Michot et al. ICAAC 2000, A662). We have examined the kinetics of accumulation and efflux of garenoxacin (GAR) and levofloxacin (LEV) and their modulation by MRP and P-gp efflux pumps, two transporters energized by ATP hydrolysis.
Methods: Accumulation in J774 macrophages at different times up to 2 h and efflux were examined at 37 °C in control cells, in ATP-depleted cells and in cells incubated with GAR or LEV in the presence of MRP inhibitors (probenecid (PRB; inhibitor of organic anions transporters) and MK-571 (preferential inhibitor of MRP)) or P-gp inhibitors (verapamil (VRP; non-specific inhibitor) and GF120918 (preferential inhibitor of P-gp)).
Results: Both antibiotics accumulated rapidly, reaching a plateau value after 5 min (cellular to extracellular ratios: GAR, 6.3 ± 0.3; LEV 3.5 ± 0.2) and their efflux was very rapid ($t_{1/2} = 1.64$ min for GAR and 2.14 min for LEV). The table shows the effect of ATP depletion and pump inhibitors on accumulation at equilibrium (2 h). PRB also decreased the rate of efflux of GAR and LEV ($t_{1/2} = 2.78$ min and 3.28 min vs. 1.64 and 2.14 min in controls, respectively) (Table 1).

Table 1

	Increase in accumulation (% of controls)	
	Garenoxacin	Levofloxacin
ATP depletion	$122.2 \pm 2.1^{***}$	$137.7 \pm 7.2^{***}$
PRB (5 nM)	$126.9 \pm 5.9^{**}$	$148.0 \pm 8.7^{***}$
MK-571 (100 μM)	$133.4 \pm 6.4^{***}$	$163.9 \pm 8.4^{***}$
VRP (100 μM)	102.7 ± 5.1^{ns}	$123.2 \pm 8.5^{*}$
GF120918 (2 μM)	102.5 ± 4.7^{ns}	110.2 ± 6.5^{ns}

ns: nonsignificant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Conclusions: GAR accumulates to a larger extent than LEV in J774 macrophages. Its accumulation is less affected than that of LEV by ATP depletion, MRP or P-gp inhibitors. Since the effect of inhibitors remains quite low for both drugs, their rapid accumulation and efflux is probably primarily mediated by their diffusion through the membrane.

P1227 In vitro release of teicoplanin from a new type calcium phosphate bone cement (Norian)

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Objectives: Norian Skeletal Repair System (NSRS) is an injectable calcium phosphate cement suitable for the structural augmentation of regions of cancellous bone cement after fractures. The aim of the study is the evaluation of the in vitro release of teicoplanin from NSRS as a means of surgical prophylaxis.
Methods: Five grams of NSRS were mixed by 100:3 ratio to teicoplanin at a room temperature under sterile conditions. The solidication of the mixture took place in the bottom of a cylindrical vial. Five samples were prepared. One mL of Mueller Hinton broth was added over the free surface of the mixture and at 37 °C temperature. Broth was replaced daily for 7 weeks (Days 1–49). Concentration of teicoplanin in broth was determined by the microbiological method using *B. subtilis* 6633 as an indicator strain.
Results: Mean concentrations of teicoplanin of five vials after 1 h was 150.8 μg/mL; after 1 day 53 μg/mL. The concentration ranged between 7.6 and 24 μg/mL on days 2–20 and 8.9 μg/mL and 24.8 μg/mL on days 21–49. Highest concentrations were detected on days 1 (53 μg/mL) and 18 (24.8 g/mL), respectively.
Conclusions: The slow release of teicoplanin (until the 49th day) from the NSRS resulted in concentrations adequately above the MICs (0.5 μg/mL) of the expected Gram-positive cocci implicated in bone infections. Supplementation of NSRS with teicoplanin should be tested in in vivo models of surgical prophylaxis.

P1228 Population PK (PopPK) characterization of the new extended-release ciprofloxacin (CIP) formulation in comparison to the immediate-release (IR) standard tablet

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Introduction: The PopPK of a novel extended release formulation for once daily (od) administration of CIP (Cipro(R) (XR) was evaluated in comparison to the previously marketed IR formulation, which is generally given b.i.d. The dissolution profile of the XR tablet with 35% immediate release followed by sustained release of the remaining dose allows it to be administered od for urinary tract infections (UTI) with the commonly known compliance benefits of a once daily treatment.
Methods: For the PK evaluation, plasma and urine concentrations from two cross-over studies in healthy male volunteers ($n = 19$ for each study) were obtained after oral intake of either a 500 and 1000 mg XR tablet or a 250 and 500 mg IR tablet bid (5 days, fasting conditions, washout period of 1 week). PopPK evaluation was performed using nonlinear mixed effect modeling (NONMEM). Two main effect compartments (urine and prostate tissue) were simulated to illustrate the target concentration exposure.
Results: The plasma PK of CIP IR and XR formulations were described by an oral two-compartment model. For ka, tlag and central volume, a random day-to-day effect (interoccasion variability) was incorporated to account for the PK complexities especially in the case of the IR tablet. The PK of the XR tablet showed lower variability between the different study/dosing occasions compared with the standard tablet. This resulted in a reduced variability of the overall PK of the CIP XR dosage form, which could be described by a first order oral two compartment model. The final model for CIP IR still showed some systemic deviations (bias) due to the higher complexity of absorption. The simulated urine concentration–time profiles were in good accord with the measured data indicating that the extended release profile translates into a sustained urine concentration–time profile exceeding the MICs of relevant UTI pathogens during the entire dosing interval. Additionally, it can be assumed that the altered plasma PK of the XR tablet will translate into similarly altered tissue PK profiles as illustrated by target tissue simulations (prostate).
Conclusions: The new XR formulation demonstrated an improved PK variability. Together with the extended release characteristics the optimized exposure–time pattern is beneficial for compliance and might be of advantage for the PK–PD relationship of CIP XR.

P1229 Teicoplanin therapeutic drug monitoring – is the thrice weekly regimen adequate for treating bone and joint infections (BJIs)?

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Background: For patients with osteomyelitis, there is evidence of a relationship between teicoplanin (TEIC) trough serum concentrations of 10 mg/L and a favorable clinical outcome. These levels are achieved by a thrice weekly

regimen of 15 mg/kg after an initial dosing regimen (15 mg/kg daily) of once daily TEIC for 3–10 days.
Objective: We have retrospectively reviewed the TEIC therapeutic drug monitoring (TDM) and clinical outcomes of 33 patients with predominantly bone and joint sepsis to assess the relationship between levels achieved by using this thrice weekly regimen with or without loading doses.
Results: Thirty-three patients, mean age 66; 18 had prosthetic infection, five had osteomyelitis, three had wound infections or complicated skin and soft tissue structure infections, six had line infections (two with bacteremia) and one a deep diabetic foot ulcer infection. 25/33 (75%) of all infections were due to MRSA (21/25) and MRSE (4/25). One infection was due to MSSA and in seven no pathogens were identified. Group a $n = 14$; all received the loading doses of 15 mg/kg for 3 days followed by 15 mg/kg thrice weekly. 13/14 has at least one trough level performed at a mean of 14 days after the start of treatment. The mean and median levels were 26.8 and 28.2 mg/L, respectively. Group b $n = 18$, all received 15 mg/kg thrice weekly without a loading dose. 18/18 has at least one trough level performed at a mean of 11 days after the start of treatment. The mean and median levels were lower at 24 and 21 mg/L, respectively. Only one 40-year-old patient had a level below 10 mg/L (8.1 mg/L) taken on day 5 and improved to >10 mg/L on two subsequent levels on day 14 and 23. In all but one patient the baseline and serial serum creatinine was within the normal range. 28/33 patients had a good outcome, in two patients there was no change or worsening and in two patients teicoplanin was stopped because of a rash or worsening renal function. In this patient the serum creatinine went up to 237 μmol/L (55–100) transiently with an associated trough level of 68.8 mg/L. This resolved after stopping the TEIC.
Conclusions: Thrice weekly TEIC with or without a prior loading regimen appears to achieve adequate serum trough levels in patients with mainly BJIs. The levels appear to be well above 10 mg L; therefore, consideration should be given to reducing the TEIC dose or loading may be unnecessary. This would further improve the cost-effectiveness of these regimens without compromising clinical outcomes.

Infection control

P1230 Would you prefer to perform endoscopy with a clean endoscope? Very good effect of a quality control program for cleaning and disinfection of endoscopes

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Introduction: Flush water from the water channel was used to evaluate a quality control program for cleaning and disinfection of flexible gastrointestinal endoscopes (FE). A clean endoscope has less than 20 CFU per ml flush water and a risk endoscope has more than 250 CFU per ml. A recent international publication found 39–49% of endoscopes ready for use contaminated at high rate (1).
Materials and methods: Three endoscopy units from three hospitals in Copenhagen Country (KAS) and six units from five hospitals in Copenhagen Hospital Corporation (H:S) participated in the program. At least 100 baseline samples were obtained immediately before an endoscopy from the different endoscopy units. Risk factors were identified, procedures for cleaning and disinfection of the endoscopes were revised and products unsuitable for cleaning and disinfection of FE were phased out. A continuous quality control program was established and included sampling before all endoscopies on a single day per month corresponding to 5–6% of all endoscopies were performed. Monthly cumulated written performance reports. Interventions when control limits were exceeded.
Results: Microorganisms isolated from risk FE were found to be either environmental/staff-related (0.3–0.74% per year) or patient-related (0.0–0.74% per year). Risk FE decreased from 1.48% in 2001 to 0.4% in 2002 ($P = 0.01$).
Conclusion:
- High quality manual cleaning is single most important factor.
- Lack of routine maintenance of washer-disinfectors leads to quality program failure.
- Ineffective disinfectants and detergents have been removed from use.
- Highest success rates were observed with energetic staff involvement.

Reference:
1. Z. Gastrointerol 2002; 40(3): 157.

P1231 Importance of biomaterial contamination duration with *Staphylococcus epidermidis* clinical isolates: pre- and post-treatment effect of hydrogen peroxide

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Porto, P

Introduction: Bacterial biofilms are commonly resistant to antimicrobial agents. The prevention of biofilm formation involves avoiding the initial step—adhesion. Hydrogen peroxide (3%) is not toxic to the human organism and should be tested as a surface treatment to prevent adhesion and growth of strains isolated from indwelling medical devices associated infections.
Objectives: to study the influence of biomaterial contamination duration with *S. epidermidis* and to assess in a pretreatment and post-treatment approach the effect of hydrogen peroxide 3%.
Materials and methods: Ninety-six-well microtiter plates were contaminated with different strains of *Staphylococcus epidermidis* (10^8 cells/mL) – RP62A, M187 (reference PS/A, +) RP62Asn, M187sn (PS/A, –) and four clinical isolates. The contamination was done over 2 and 6 h at 37 °C in saline solution (0.9%) (1). After contamination, 96-well microtiter plates were rinsed with saline solution to remove nonadherent bacteria. A modified microtiter-plate assay was used as a direct measure of adherence (2). A pre- and post-treatment with a saline solution and hydrogen peroxide was also assayed.
Results: (Figure 1) A pretreatment with saline (0.9%) solution, when contamination period was 2 h, reduced bacterial adhesion but no reduction was found with 6 h. In contrast, 3% hydrogen peroxide was always effective, irrespective of the duration of contamination.

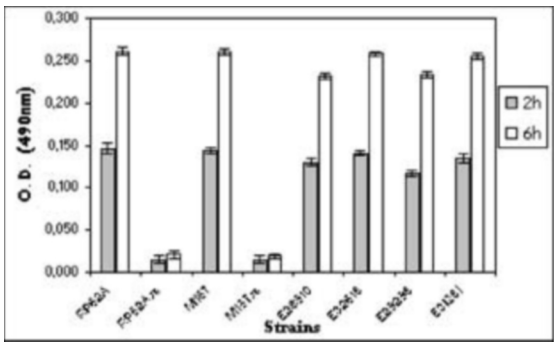

Figure 1

Conclusions: The biomaterials contamination duration significantly increases adhesion values mainly in PS/+strains. Hydrogen peroxide was more efficient in prophylactic effect than saline solution after a 2-h contamination. The effect of saline solution and 3% hydrogen peroxide on adhesion was identical in all *S. epidermidis* strains. In post treatment, saline (0.9%) solution had a visible effect on bacteria disattachment after a 2 and 6 h contamination period, but its effect was more evident after 2 h contamination, where adhesion values were almost identical to the values obtained with hydrogen peroxide. The effectiveness of saline solution was reduced when the contamination period was prolonged to 6 h. Hydrogen peroxide (3%) was always effective, after 2 and 6 h, like in pretreatment.
Acknowledgement: This study was supported by a research grant from Fundação Calouste Gulbenkian.

References:
1. Alt *et al.* Ann. Thorac. Surg. 1999; 68: 2123–8.
2. O'Toole *et al.* Mol. Microbiol. 2000; 30: 285–93.

P1232 Relevance of interval between biomaterial treatment with hydrogen peroxide and contamination with different strains of *Staphylococcus epidermidis*

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Introduction: Bacterial biofilms are sessile communities to the ability to resist antimicrobial agents. Prevention of such communities involves avoiding the first step, which is adhesion. Hydrogen peroxide in 3% is not toxic to the human organism and should be tested in strains isolated from indwelling medical devices associated infections.
Objectives: To study the influence of the sequence of disinfection and contamination as well as of the persistence of hydrogen peroxide effects on the prevention of biofilm formation.
Materials and methods: 96-well microtiter plates were contaminated with strains of *S. epidermidis* (10^8 cells/mL), RP62A, RP62Asn, M187, M187sn—that were used like controls—and four clinical isolates. The contaminations were done over 2 and 6 h at 37 °C in saline (0.9%) solution. After contamination, 96-well plates were rinsed saline solution to remove nonadherent bacteria. A modified microtiter-plate assay was used as a direct measure of adherence (1). The interval between washing with saline (0.9% NaCl) solution and 3% hydrogen peroxide was prolonged to 16 h (2). In order to achieve this aim, a wash procedure with saline and hydrogen peroxide solution was effectuated 16 h before a 2-h/6-h contamination.
Results: There was no protective effect of saline solution for contaminations longer than 16 h after treatment. There was a diminution on adhesion values, when the interval between treatment and contamination was null, for a 2-h contamination period, but it was not valid when the gap becomes 16 h. In contrast, pretreatment with 3% hydrogen peroxide had diminished adhesion of all different strains used. Adhesion values were very close to those ones observed to a null interval between washing and contamination.
Conclusions: Hydrogen peroxide (3%) is effective as a prophylactic treatment in order to avoid bacterial growth after contamination. Randomized, prospective clinical studies are required to substantiate the importance of in vitro findings, as far as these particular clinical *S. epidermidis* strains of nosocomial origin are concerned.
Acknowledgement: This study was supported by a research grant from Fundação Calouste Gulbenkian.

References:
1. O'Toole *et al.* Mol. Microbiol. 2000; 30: 285–93.
2. Alt *et al.* Ann. Thorac. Surg. 1999; 68: 2123–8.

P1233 Study of *Salmonella* contamination of foods in Isfahan, Iran

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Objective: To determine the prevalence of *Salmonella* on raw and cooked foods from selected university restaurant.
Methods: Two hundred and sixteen food samples were obtained randomly in Isfahan University of Medical Sciences between April and June 2002. All were tested for the presence of *Salmonella* using enrichment method (Iranian standard method of isolation of salmonella no. 1081).
Results: *Salmonella* detected in 21 and 7% of raw and cooked poultry samples, respectively. On the samples of red meats 12.5 and 2.7% were contaminated by *Salmonella*, respectively. Nine percentage of vegetable were found to be contaminated by *Salmonella*. All samples taken from fish and milk products were free of *Salmonella*.
Conclusions: *Salmonella* spp. are present in a small proportion of cooked foods and are not killed during cooking process or the contamination may occur after thermal treatment. These finding have clear implication for public health and indicating the needs for improvement of hygienic condition in food processing centers.

P1234 Evaluation of anti-*Toxoplasma gondii* antibodies (IgG and IgM) in sera among the women before marriage in Urmia city, Iran

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Introduction: Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, and obligate intracellular parasite. It is primary host are cats and in man infection occurs mainly by ingestion of raw or underdone meats and vegetables. The most significant infections in humans are congenital and during first semester of pregnancy cause abortion. Immunologic methods are the most common way to establish the diagnosis. The aim of this study was to determine the incidence of IgG and IgM anti-*Toxoplasma* antibodies in women before marriage in Urmia city Iran.
Material and methods: Blood of 300 women from urban and rural areas before marriage were collected. Anti-*Toxoplasma* IgG and IgM antibodies were determined from sera by using ELISA and IFA techniques.
Results: Anti-*Toxoplasma* IgG antibodies were found positive in 98 (32.8%) and anti-*Toxoplasma* IgM antibodies were found positive in 12 (4%) of these samples by ELISA method. The same samples were examined by using indirect IFA technique. The IgG titer in 65 samples was 1/400, 13 samples 1/800, 12 samples 1/1600 and four samples 1/3200 and four samples showed negative results.
Discussion: The positive results were indicated 4% of these women suffer from acute Toxoplasmosis and 28.8% of women suffer from subacute or chronic Toxoplasmosis. There were not statistical significant differences between those from rural and urban areas. As a result, the control of disease in both area is recommended. The details will be discussed at the conference.

P1235 Immunology, epidemiology and prognosis of tetanus: a retrospective study

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Objectives: Tetanus is a toxi-infectious disease which can be prevented by vaccination, having high mortality, and the follow-up and treatment of which requires hard work. The aim of this study is to investigated retrospectively in view of epidemiologic features, immunization history, clinical features, prognosis and treatment.
Methods: Forty-seven patients, 46 generalized and one localized, who were followed-up and treated with the diagnosis of tetanus between January 1985 and December 2002 in Infectious Diseases Clinic, Faculty of Medicine, Atatürk University, were investigated retrospectively. The data concerning the patients were obtained through the investigation of the files. The places where the patients lived, their occupations, age, set, immunization history, infection site and the type of injury, the prophylactic approach after injury, incubation period of the disease, clinical features, prognosis and treatment applications were recorded.
Results: Forty-four (94%) of the cases, 31 male and 16 female were from rural areas. The rate of men (66%) was approximately twofold than the rate of woman. Fifty-four percentage were over 50 years old. Fifty-four had minor injuries. Appropriate tetanus prophylaxis had not been given to nine of the cases who applied to health centers after injury. Average incubation period was 11.1 days, and the most common symptom and finding was difficulty in swallowing and trismus. Mortality was 40.4%, and the most features that affected prognosis were age and incubation period. The difference of mortality between men and woman was not significant ($z = 0.27$, $P = 0.39$).
Conclusions: The result showed that adult immunization against tetanus was not adequate in the east of Turkey, especially in rural areas, and health personnel do not perform adequate immunization after tetanus-prone injuries. We believe that application of adult immunization programme and training of health personnel in applying adequate prophylaxis protocols will decrease tetanus incidence.

P1236 Etiology and incidence of halitosis in Latvia

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Objectives: Oral bacteria hydrolyze proteins and further degrade the amino acids, which leads to halitosis, commonly referred to as bad breath. Halitosis is a frequent problem in Latvia. The goal of our investigation was to state etiology, diagnostic and treatment methods of halitosis.
Methods: The recent investigation began in 1997 and included 578 patients of dental multidisciplinary outpatient department. A detailed patient's questionnaire was prepared. It covered questions about the use of antibiotics and other medicine, affecting the quality and quantity of saliva, as well as questions concerning smoking, the use of alcohol, etc. The oral odor or bad breath was confirmed by the measurements made by the halimeter (Interscan Corporation, Model RH-17E USA). The halimeter quantifies breath measurements

in parts per billion (p.p.b.) of volatile sulfur compounds (VSC). The obtained results were compared with dental (DMF index) and periodontal (PMA, CPITN indexes) conditions.
Results: The measurements of the halimeter correlated with the clinical assessment of breath odor, the degree of tongue coating and the depth of the gingival pockets. 578 patients were examined. It was determined that the main cause of halitosis is oral pathology (89%). From which 53% were observed due to the tongue coating. It was concluded from the patient's questionnaire that increased level of tongue coating is due to a short-term or frequent use of antibiotics. 39% of oral pathology derives from periodontal diseases (severe periodontal diseases—periodontitis, 31% and gingivitis, 8%.). In remaining 8%, a complex oral pathologies were diagnosed—tongue coating and gingivitis, tongue coating and periodontitis. The halimetric measurements of patients of this group showed a higher amount of VSC, in addition, the duration of the preceding therapy of these patients was longer.
Conclusions: The results of the recent examination leads to following conclusions: halitosis in Latvia is mainly connected with oral pathology; halitosis can be diagnosed using different methods (especially halimetric examinations) and can be treated successfully; protein degradation in oral cavity and increased amount of VSC is closely connected with the changes of oral microflora (with a prevalence of oral anerobes), this leads to different periodontal diseases, which are rather common in Latvia.

Antituberculosis chemotherapy and susceptibility testing

P1237 Determination of *Mycobacterium tuberculosis* drug sensitivity in Mazandaran province health center in 2002

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Objectives: Tuberculosis (TB) is one of the most important infectious disease and the main cause of mortality in the developing countries. There have been educational program in order to prevent it, not only has not been eradicated in the recent years, but also has an increasing rate in the HIV patients. Drug resistant leads to failure in treatment which is followed by unaffordable expenses. Hence, study on the drug sensitivity of such organism provides guideline on the pattern of epidemiology study, as a result help reduce the episode of drug resistant species in the society.
Materials and methods: Sputums from the individuals referring to health center suspected of having TB were collected in sterile containers, decontaminated by PETROPH method and contracted, then inoculated on to the Levenson jansons medium and incubated at 37 °C for 4 weeks. Smears were prepared from the collected samples zeihl neelson staining was performed and looked for the presence of mycobacterium. Culture media were observed for the growth of *Mycobacterium tuberculosis* (TB). The culture positive plates were sent to Masih daneshvari TB center in Tehran, to be confirmed for the growth of TB and doing antibiogram.
Results: In this study, on 45 TB growth cultures, drug sensitivity was done for four essential drugs that is isoniazid (INH), refampin (Ref), ethambotol (Eth) and stereptomycin (ST) which are used against TB. The pattern of sensitivity from 45 samples are as follows: Eth 44 samples sensitive and 1 sample resistant (2.2%), INH 40 samples sensitive and 5 resistant (11%), Ref 43 samples sensitive and 2 resistant (4.4%), ST 31 samples sensitive and 14 resistant (31%) and was separately 4 to 1, and combined resistant to two drugs that is INH and ST 2 samples and to three drugs that is to INH and Eth and ST 1 sample.
Conclusion: The results of this study indicate that, resistant to Eth, INH, Ref and ST is 2.2, 11, 4.4 and 31%, respectively, which reveals an increasing pattern of TB drug resistant. Hence, increase in educational training of the physicians and paramedical personals for the proper short course treatment and implementation of directly observed treatment short course (Dots) strategy considered as prior issue is proposed. Also determining of drug sensitivity which is not common in our country is recommended in order to prevent the recurrent of the disease and failure of treatment.

P1238 Investigation of susceptibilities to primer antituberculous agents of *Mycobacterium tuberculosis* complex strains isolated in Manisa, Turkey

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Manisa, TR

Objective: In this study, we aimed to investigate the susceptibilities of *Mycobacterium tuberculosis* complex isolates in Manisa region to isoniazid, rifampicin, streptomycin and ethambutol.
Methods: One hundred and twenty-nine strains which were isolated from respiratory system specimens between 1997 and 2002 were evaluated in Celal Bayar University Faculty of Medicine, Mycobacteriology Laboratory. BACTEC radiometric method and indirect proportional method in Lowenstein Jensen medium were used for testing the susceptibilities.
Results: Eighty-eight strains (68.2%) were susceptible to all tested agents, and the resistance rates of streptomycin, isoniazid, rifampicin and ethambutol were found as 20.2, 17.1, 12.4 and 7%, respectively. The strains which were resistant to at least one agent, two agents and three agents were determined as 17.8, 6.2 and 4.7%, respectively. The rate of resistance to all agents was also 3.1%. Multiple drug resistance was found in 11 strains (8.5%). Although it was not found to be statistically significant, it was determined that the frequency of rate rifampicin resistance and multiple drug resistance was increasing by years. There was an increasing rate of rifampicin and multidrug resistance year by year, although not statistically significant.
Conclusions: The determination of increased rate of rifampicin and multiple drug resistance should led us to use reliable and rapid methods in evaluating the susceptibility rates of antituberculous agents.

P1239 Early bactericidal activity and efficacy of moxifloxacin vs. isoniazid in the treatment of acute pulmonary tuberculosis—a prospective, randomized study

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Objective: In several in vitro studies Moxifloxacin was demonstrated to be the currently most active fluorquinolone against *Mycobacterium tuberculosis* (Mtb). However, data about the efficacy in patients are not yet available. The decrease of colony-forming units (cfu) of Mtb in sputum and the early bactericidal activity (EBA) of Moxifloxacin were compared with those of INH in a prospective, open, randomised, monocentric study.
Methods: Fourteen adult, not immunocompromised patients with an active, smear positive, pulmonary tuberculosis were treated with either Moxifloxacin (400 mg/day; MOX, $n=7$) or Isoniazid (5 mg/kg body weight; INH, $n=7$) for 5 days and followed by a standard therapeutic regimen. EBA was defined as the fall in log counts cfu/mL sputum/24 h during the first 2 days.
Results: After 5 days a significant decrease of median values of CFU/mL was detected in both groups (INH: day $1=9.9\times10^6$/mL, day $6=0.58\times10^6$/mL, $P=0.043$; MOX: day $1=8.2\times10^6$/mL, day $6=0.60\times10^6$/mL, $P=0.046$). There was no significant difference between both groups regarding the EBA (MOX = 0.453, INH = 0.598). The overall tolerance of study medication in both groups was good.
Conclusion: According to the limited data of our study Moxifloxacin exhibits an in vivo efficacy that is almost comparable to that of INH.

P1240 Evaluation of second-line antitubercular drugs for *Mycobacterium tuberculosis* using the BacT Alert 3D system

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Objectives: (a) Isolate and identify isolates of *Mycobacterium tuberculosis* from pulmonary and extra pulmonary specimens using routine culture media and the BacT Alert 3D System. (b) Perform antimicrobial susceptibility testing using first and second line antitubercular drugs.
Material and methods: One hundred isolates of *M. tuberculosis* recovered from pulmonary and extra pulmonary specimens were studied. These were subjected to antibiotic susceptibility testing using the BacT Alert 3D System (bioMerieux, France); the results were reconfirmed using the E-strip method.

The following antimicrobials were tested: First Line: streptomycin, isoniazid, rifampcin, ethambutol, pyrazinamide. Second line: ethionamide, ciprofloxacin, ofloxacin, amikacin, capreomycin and kanamycin.
Results: Combined resistance to isoniazid and rifampicin (Multi Drug Resistance) was observed in 11 (11%) isolates. Single drug resistance was seen in 63 (63%) of the Mycobacteria. Isoniazid resistance was observed to be the commonest, followed by ethambutol, streptomycin, rifampcin and pyrazinamide. Quinolones and amikacin were found to be the most effective agents, followed by kanamycin and capreomycin. The tubercle bacilli recovered from extra pulmonary sites were found to be more sensitive than the pulmonary isolates.
Conclusions: (i) It was observed that multi drug resistant strains are being increasingly isolated from lesser developed nations, especially from the urban areas. (ii) The isolates are showing resistance to the commonly employed second line antitubercular drugs. Resistance to second line drugs has not been well documented previously. With the raging triple epidemic of HIV, STD and TB, this fact could have far reaching consequences.

P1241 Determination of the susceptibilities to major antituberculous drugs by *E*-test method of 45 *Mycobacterium tuberculosis* isolated from cerebrospinal fluid

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Objective: Tuberculosis keeps its importance since it has an increasing frequency in the society besides its increasing resistance rates. Tuberculous meningitis is one of the most serious forms of this disease. In this study, we aimed to examine the susceptibility of *Mycobacterium tuberculosis* strains, isolated from cerebrospinal fluids of patients with tuberculous meningitis, to four major drugs.
Methods: 1515 CSF specimens were sent to our laboratory through the years 1992–2002. 142 of them had a growth on Lowenstein-Jensen medium. For the detection of the species; niacin test, nitrite test, catalase test, catalase test after heating to 68 °C had been held. The MICs of 45 M tuberculosis strains for isoniazid (INH), rifampicin (RIF), ethambutol (ETB) and streptomycin (SM) were determined by *E*-test method.
Result: The MIC values of the examined strains reflect that the resistance rates are very low. The MIC 90 values for INH, RF, ETB and SM were detected as 0.016, 0.016, 0.023 and 0.125 μg/mL, respectively. In one case, resistance to four antituberculous drugs has been detected. There was no other resistant strain.
Conclusion: Although the resistance rates isolated from CSF have been found to be low, since tuberculosis meningitis is threatening public health and the resistance rates are increasing in years, antibiotic susceptibility tests should be studied for all strains.

P1242 A general view for resistance problem: the MIC values of 215 *Mycobacterium* spp. against major antituberculous agents by *E*-test

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Objective: Among the materials that were sent to Istanbul Haseki Education and Research Hospital microbiology laboratory, 215 acid-fast bacilli isolated from Lowenstein-Jensen medium were defined as *Mycobacterium tuberculosis* and were investigated for their antituberculous sensitivity.
Methods: Sputum, cerebrospinal fluid and abscess constituted most of the materials. Abscess had the highest growth rate with a percentage of 16%. MIC values were examined with *E*-test method for isoniazid, rifampicin, ethambutol and streptomycin in Middle Brook medium.
Result: The resistance ratios for the species were found to be very low. The MIC 90 ratios for isoniazid, rifampicin, ethambutol and streptomycin were found to be 0.016, 0.016, 0.032 and 0.064 μg/mL, respectively, implying sensitivity. The resistance rates for isoniazid and ethambutol were found 2.33% whereas for rifampicin and streptomycin were 1.86%. The resistant strains were mostly isolated from sputum and CSF. The resistance for the all four antituberculous drugs were found in only two patients.
Conclusion: Resistance researches have to be carried out for all species to continue the fight against tuberculosis that still threatens the public health seriously even in today's world.

P1243 Comparison of Dio-TK and Löwenstein-Jensen media in antimycobacterial susceptibility testing for *Mycobacterium tuberculosis* by using the proportion method

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Objective: To evaluate Dio-TK Medium in antimycobacterial drug susceptibility testing for *M. tuberculosis* strains in comparison with Löwenstein-Jensen (L-J) medium.
Methods: A total of 78 *M. tuberculosis* recovered from the positive cultures of 69 patients were taken into the study. Bacterial suspension(100 μL) was inoculated in Dio-TK Medium, Dio-TK INH (Isoniazid 0.2 and 1 μg/mL), Dio-TK RIF (Rifampicin 1 μg/mL), Dio-TK ETB (Ethambutol 15 μg/mL) and Dio-TK STR (Streptomycin 2 μg/mL). Growth and resistance on Dio-TK Medium was monitored and evaluated with the color change from red to yellow due to the indicator in the medium by the automated incubator reader Dio-TK Scan and presence of acid-fast bacteria on the medium by staining. 10–2 dilutions of suspension were inoculated into L-J medium and L-J media with drugs (INH 0.2 and 1 μg/mL, RIF 40 μg/mL, ETB 2 μg/mL, STR 4 μg/mL). If the ratio of growth (cfu) in the media including drug to the growth in the media without drug was higher than 1%, the strain was accepted as resistant.
Results: Resistance ratios for INH, RIF, ETB, STR in Dio-TK medium were 24.6, 22.6, 22.9 and 21%, whereas; ratios in L-J medium were 18.3, 15.6, 11 and 16.6%, respectively. Contamination ratios of Dio-TK with INH, RIF, ETB and STR were 5, 3.8, 5 and 3.8%; whereas ratios in L-J were 16.6, 11.5, 11.5 and 12.8%, respectively. All the isolates susceptible to four drugs in Dio-TK were also susceptible in L-J. Five of the specimens from patients did not grow in L-J medium, therefore drug susceptibilities of those only were studied in Dio-TK Medium. Two of these isolates were resistant to all four drugs, one was susceptible to all drugs and the other two were resistant to RIF and ETB together. Mean time of growth for Dio-TK and L-J were 10.6 (minimum 4, maximum 20) and 20 (min.15, max. 25) days, respectively.
Conclusions: Dio-TK Medium is a rapid and practical medium and may be very beneficial in obtaining antimycobacterial susceptibility testing results early. The concentration of some antituberculosis drugs may require modifications for a better concordance with classical tests.

P1244 Susceptibility patterns of *Mycobacterium* spp. isolated in a teaching hospital in Madrid during a 3-year period (2000–2002)

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Objective: The aim of this work was to study the susceptibility patterns of *Mycobacterium* spp. to the antimicrobials most frequently used in their treatment, isolated in the Hospital Universitario de La Princesa in the period of 2000–2002.
Methods: A total of 146 clinical isolates were included in this study: 130 *M. tuberculosis* (105 from respiratory samples and 25 from non respiratory), 4 *M. avium* complex (MAC), 9 rapidly growing mycobacteria (4 *M. fortuitum*, 4 *M. chelonae*, and 1 *M. abscessus*) and 3 *M. kansasii*. Identification was obtained by a DNA probe (Accuprobe, Gen Probe) for *M. tuberculosis* and MAC and the rest were identified in a Reference Mycobacterial Laboratory. Susceptibility testing was performed by SIRE (Becton Dickinson), a fluorescent, non radiometric mycobacterial broth system in the case of *M. tuberculosis*, Radiometric BACTEC TB 460 (Becton Dickinson) for MAC and *M. kansasii* and broth microdilution for rapidly growing mycobacteria. Antimicrobial agents studied were: streptomycin, isoniazid, rifampin, ethambutol and pyracinamide for *M. tuberculosis* and *M. kansasii*, macrolides, quinolones, aminoglicosides and tetracycline for rapidly growing mycobacteria and rifabutin was added for MAC.
Results: Those related to *M. tuberculosis* were as follows: 16 (12.3%), 7 (5.38%), 3 (2.3%) and 5 (3.8%) were resistant to streptomycin, isoniazid, rifampin and ethambutol, respectively. All the isolates tested (21) were susceptible to pyracinamide. Three (2.3%) strains were resistant to both streptomycin and isoniazid, 2 (1.5%) to isoniazid plus rifampin, 1 (0.7%) to streptomycin plus ethambutol and 1 (0.7%) isolate was resistant to all drugs. 3 out of 4 (75%) MAC strains were resistant to ofloxacin and clarithromycin and 2 (50%) were resistant to both. All of them were susceptible to rifabutin.

Rapidly growing mycobacteria showed the following susceptibility pattern: 55.5% resistance to ciprofloxacin, 33.3% to clarithromycin, 78% to doxicicline and 12.5 to amikacin. All *M. kansasii* were susceptible to rifampin and ethambutol and resistant to streptomycin and isoniazid.
Conclusion: We did not find an important number of multidrug *M. tuberculosis* strains. According to the variability in the susceptibility pattern of rapidly growing mycobacteria, susceptibility test should be performed when these mycobacteria are related with clinical significance.

P1245 Susceptibility testing of *Mycobacterium tuberculosis* by flow cytometry

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Background: *Mycobacterium tuberculosis* is a re-emerging agent and represents an important cause of human disease. The detection of multidrug resistance in clinical strains urged the effort to develop rapid tests to detect susceptibility patterns. Cytometric methods show great potentialities in the area of Microbiology, by giving faster and accurate results than classical susceptibility methods.
Objective: To determine the susceptibility of *Mycobacterium* using the fluorescent probe SYTO 16 and flow cytometry, comparing to culture with BD BACTEC MGIT 960 system protocol (Becton Dickinson Company, Maryland, USA).
Material and methods: Eight strains of *M. tuberculosis* belonging to a Portuguese multicentric quality control program were studied using BD BACTEC MGIT 960 protocol. They were classified as sensitive, intermediate and resistant to four drugs: ethambutol, isoniazid, rifampicin and streptomycin (SIRE). In this study, we performed incubation of the strains in MGIT tubes, using SIRE kits (BD), in duplicate. One set was incubated during 72 h at 37 °C while the other followed the BACTEC MGIT protocol (10–14 days of incubation). Following incubation, both sets of MGIT tubes were autoclaved, to assume microbiologic safety of the cytometric study, and stained with 10 μM of SYTO 16 (molecular probes), a fluorescent nucleic acid stain during 30 min. A ratio between the number of microbial cells with fluorescence and the control (cells not exposed to drug) was calculated. Sensitivity represents a strain showing a ratio <1 (meaning a reduction of cells on drug tube in comparison with control). Intermediate strains present a ratio ≥ 1 at the highest concentration of the drug and <1 at the lowest concentration. Resistant strains show a ratio ≥ 1, both with low and high concentrations. A control of contamination was performed by staining the cells in each tube with propidium iodide (PI) 1 μg/mL, for 30 min (Sigma).
Results: The results of the cytometric method were equivalent, both after 72 h or 10–14 days incubation. The agreement between cytometric methods and BACTEC 960 protocol was 100%. The extent of contamination was similar in both methods, being revealed in cytometry by the fact that only microbial cells, other than Mycobacterium stained by PI.
Conclusion: The flow cytometric assay described is a safe, fast, simple and reproducible assay to be used in sensitivity testing of *M. tuberculosis*.

P1246 Real-time PCR assay for detection of MDR *Mycobacterium tuberculosis*

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Background: The emergence of multidrug resistant strains of *Mycobacterium tuberculosis* (MDR-TB) strengthens the need for routine determination of drug susceptibility of clinical isolates in order to establish an efficient therapeutic treatment of the patient. The traditional methods for susceptibility testing in mycobacteria rely on culture-based techniques, which are extremely time consuming due to the low growth rate of the microrganism.
Objectives: To set up a molecular assay able to rapidly detect the mutations most frequently associated to the drug resistance to isoniazid and rifampin in *M. tuberculosis*.
Methods: Hybridization Probes were designed to differentiate wild type codons from mutated ones, in real time PCR. Two different PCR assays were set up, one for the detection of Isoniazid resistance and the other one for rifampin resistance. Target genes were katG and rpoB. Probes were designed on those codons shown to be mutated in a molecular epidemiology study on Italian MDR-TB isolates. Probes were validated on a collection of 53 Italian MDR-TB isolates.
Results: The association of both PCR reactions allows us to identify resistant alleles in 94.2% of MDR-TB strains (49/53).
Conclusion: Real-time PCR based on the hybridization probes is a rapid and reliable system to perform a molecular antibiogram in *M. tuberculosis* MDR strains. The source for the susceptibility test may be either a smear positive specimen or a positive primary culture. This molecular assay permits to obtain a preliminary and essential information on drug susceptibility of the isolate within few hours from the specimen collection, while about 4–6 weeks are necessary for culture based determination of resistance.

P1247 A 10-year monitoring of in vitro antimicrobial susceptibility levels of *Mycobacterium xenopi* as an agent of HIV-associated vs. non-HIV-associated disease

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Objective: Aim of our study is to assess the epidemiology and in vitro susceptibility of 35 consecutive *Mycobacterium xenopi* strains responsible for confirmed disease at a reference centre in the decade 1993–2002, and to identify differences between the in vitro sensitivity of the 17 strains isolated from 14 patients with HIV disease, and the 18 isolates cultured from 18 non-HIV-infected patients.
Results: Compared with non-HIV-infected patients, HIV+ patients had a lower mean age ($P < 0.0001$) and a tendency to develop long-term relapses ($P = 0.06$). Gender distribution and *M. xenopi* disease site did not show differences between the two study groups. Lower airways accounted for 88.6% of cases, but a prominent inflammatory reaction recently emerged in HIV-infected patients favorably treated with HAART, raising the role of the rapid immune recovery in the pathomorphism of this opportunistic disease. The greatest in vitro sensitivity rate was registered by capreomycin and protionamide (100% of strains), followed by kanamicin (96.6%), while susceptibility rates for the usual 1st-line compounds like ethambutol, isoniazid and rifampicin were slightly lower (86–91%). No temporal variation in susceptibility was seen over the study decade, while non-HIV-infected patients had a higher frequency of *M. xenopi* isolates resistant to >1 compound ($P < 0.03$), versus HIV-associated episodes, despite the heavy and prolonged exposure of HIV-infected patients to antimicrobials, including agents acting on mycobacteria. When considering the different antimycobacterial compounds, resistance proved slightly more frequent among non-HIV-infected patients. Only one HIV-positive patient became rifampicin-resistant in his 3rd relapse.
Conclusion: Diagnostic difficulties due to late identification and concurrent opportunism, add to therapeutic problems due to a unpredictable sensitivity pattern of atypical mycobacteria, such as *M. xenopi*. Surprisingly, 2nd-line antimycobacterial compounds (capreomycin, protionamide and kanamicin), demonstrated a slightly greater activity than 1st-line drugs recommended for *M. xenopi* infection (ethambutol, isoniazid, rifampicin-rifabutin, and streptomycin). A rapid identification, a reliable differentiation between colonization and disease, and treatment choice for atypical mycobacteriosis, are still unresolved issues for clinicians and bacteriologists treating immunocompromised patients. The emerging resistance to traditional antimycobacterial agents warrants extensive studies with promising agents (macrolides, quinolones), while also older drugs may act favorably.

Streptococcus pneumoniae

P1248 Comparative activities of beta-lactam antibiotics and quinolones for invasive *Streptococcus pneumoniae* isolates

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Objectives: *Streptococcus pneumoniae* is a leading pathogen causing pneumonia, meningitis, otitis media, bacteremia, sinusitis and a significant cause of morbidity and mortality. The rapid emergence of penicillin nonsusceptible and multidrug resistance strains of *S. pneumoniae* has encouraged the development of new antimicrobial agents such as quinolones with increased activity against *S. pneumoniae*. In this study, we examined in vitro activities of five quinolones in comparison with other antibiotics for 85 invasive pneumococcal isolates (derived from bacteremia, pneumoniae and meningitis cases between January 2000 and December 2001) collected in the hospital of Istanbul Medical Faculty.
Methods: MICs of penicillin G, cefuroxime, azithromycin, clarithromycin, trimethoprim-sulfamethoxazole (SXT), ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin and gemifloxacin were determined by the NCCLS recommeded broth microdilution testing method in Mueller-Hinton broth supplemented with 2% freeze-thaw lysed horse blood with an inoculum of approximately 105 cfu/mL.
Results: The overall rates of resistance to penicillin (46%), cefuroxime (20%), azithromycin (20%), clarithromycin (18%) and SXT (46%) were considerable. Among all of the isolates, nine isolates (11%) were highly resistant (MIC, ''d 2 mg/L) and 30 isolates (35%) had intermediate resistance (MIC, 0.12–1.0 mg/L). Of the fluoroquinolones investigated for their activity against *S. pneumoniae*, gemifloxacin (MIC90 = 0.12 mg/L) and trovafloxacin (MIC90 = 0.12 mg/L) had the highest activity. These values were three dilutions lower than those obtained with levofloxacin (MIC90 = 1 mg/L), four dilutions lower than those obtained with ofloxacin (MIC90 = 2 mg/L) and ciprofloxacin (MIC90 = 2 mg/L). Status of penicillin resistance apparently did not have any affect in the quinolone resistance of the isolates.
Conclusion: The new fluoroquinolones show great potential for treating infections caused by penicillin-resistant pneumococci. Resistance to macrolides and to another beta-lactam antibiotic, cefuroxime, was more often found among penicillin-resistant isolates. This observation limits the value of these antibiotics for empirical treatment of pneumococcal infections in geographic areas where penicillin-resistant *S. pneumoniae* are widespread. The broad-spectrum antimicrobial activity of these new quinolones indicates these compounds may be useful in the empirical treatment of pneumococcal infections.

P1249 Penicillin-nonsusceptible *Streptococcus pneumoniae* in Europe and worldwide: susceptibility to amoxicillin/clavulanic acid, including a new pharmacokinetically enhanced formulation, in 2001. The Alexander Project

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Objectives: The Alexander Project is a prospective surveillance study of antimicrobial susceptibility. Susceptibility of penicillin-nonsusceptible *Streptococcus pneumoniae* (PNSP) to amoxicillin/clavulanic acid (AMX/CA) at the NCCLS breakpoint (≤2 mg/L, susceptible) was assessed. A susceptible pharmacokinetic/pharmacodynamic (PK/PD)-based breakpoint of ≤4 mg/L for a new, pharmacokinetically enhanced formulation of AMX/CA (2000/125 mg BID) has been proposed; susceptibility at this breakpoint was also determined.
Methods: Respiratory isolates were collected from centres worldwide in 2001. MICs and susceptibility to AMX/CA for isolates of *S. pneumoniae* determined to be penicillin-nonsusceptible (penicillin MICs ≥0.12 mg/L) were determined according to NCCLS methodology and breakpoint (AMX/CA susceptible breakpoint ≤2 mg/L). Susceptibility to AMX/CA 2000/125 mg BID was also determined at a PK/PD-based breakpoint of ≤4 mg/L.
Results: Percentage susceptibility for isolates collected in Europe and worldwide is given in the table.

%PNSP susceptible to AMX/CA in 2001 (n/N)

Europe		Worldwide	
≤2 mg/L	≤4 mg/L	≤2 mg/L	≤4 mg/L
88.5 (246/278)	93.5 (260/278)	88.4 (733/829)	94.3 (782/829)

Susceptibility to AMX/CA at a breakpoint of ≤2 mg/L (conventional formulations) among isolates of PNSP was below 90.0% in Europe and worldwide in 2001. Using the proposed PK/PD-based breakpoint of ≤4 mg/L for the new formulation, however, susceptibility was above 93.0% in Europe and worldwide.
Conclusions: The new pharmacokinetically enhanced formulation of AMX/CA has an extended time above MIC, which supports the use of the proposed breakpoint of ≤4 mg/L, and may therefore be a useful option for empiric treatment of respiratory infection in areas where PNSP are prevalent, and where susceptibility to conventional AMX/CA has decreased.

P1250 Decreasing *Streptococcus pneumoniae* susceptibility tomacrolides in five European countries: the Alexander Project

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Objectives: The Alexander Project is a prospective surveillance study of respiratory pathogen susceptibility to antimicrobials. Trends in *Streptococcus pneumoniae* susceptibility to macrolides over 10 years in five European countries were analyzed.
Methods: Isolates were collected from centers in France, Germany, Italy, Spain and the UK annually in 1992–2001. *S. pneumoniae* MICs and susceptibility to erythromycin (ERY), clarithromycin (CLA) and azithromycin (AZI) were determined using NCCLS methods (ERY, CLA susceptible breakpoint ≤0.25 mg/L; AZI susceptible breakpoint ≤0.5 mg/L).
Results: Percentage susceptibility to ERY for each year and country are given in the table.

	% susceptible									
Country	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
France	74.0	65.3	56.1	60.9	59.4	54.1	57.1	43.9	41.4	43.6
Germany	99.0	100	93.9	97.3	97.3	93.5	94.3	93.7	91.7	91.2
Italy	98.6	95.1	84.8	81.0	75.9	70.2	58.0	68.6	67.6	64.1
Spain	90.0	87.1	74.8	74.0	80.9	67.4	80.6	64.8	71.3	73.6
UK	97.6	96.7	98.0	94.4	86.4	92.8	82.4	90.7	88.7	88.5

All five countries showed a decrease in *S. pneumoniae* susceptibility to all macrolides tested over the 10-year period. In 1992, only France had macrolide susceptibility rates below 90%. By 2001, macrolide susceptibility in all countries but Germany had fallen below 90%, with the greatest decrease in susceptibility seen in Italy. Of isolates that were resistant to erythromycin in 2001, 94.6% (88/93) (France), 81.8% (9/11) (Germany), 75.7% (28/37) (Italy), 100% (28/28) (Spain) and 50% (5/10) (UK) were also resistant to clindamycin.
Conclusions: In all five European countries studied, there was a trend towards decreased susceptibility of *S. pneumoniae* to all macrolides tested from 1992 to 2001. ERY cross-resistance with clindamycin seen in 2001 indicates that the majority of isolates possess the MLS(B) phenotype. Combined with a downward trend in susceptibility, this suggests that the effectiveness of macrolides in the clinical setting may be limited, and this should be taken into account when making prescribing decisions to optimize treatment outcomes. Continuing surveillance is needed to monitor further changes in respiratory pathogen susceptibility.

P1251 Trends in single or multiple antimicrobial resistance phenotypes among *Streptococcus pneumoniae* collected worldwide: 1999–2002

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Objectives: Multidrug-resistant (MDR) *S. pneumoniae* (SP) are associated with increased patient and hospital costs, higher patient mortality rates, and fewer therapeutic choices for physicians. MDR phenotypes are becoming more prevalent, and continued surveillance is necessary to monitor changes in MDR as they occur.
Methods: In 1999–2000 ($n = 4951$) and 2001–2002 ($n = 5742$), SP were isolated at hospitals in 12 countries and tested by broth microdilution against penicillin (PEN), levofloxacin (LEV), azithromycin (AZI), ceftriaxone, trimethoprim-sulfamethoxazole (TMP-SMX), and comparator agents.
Results: Globally, there was no significant change (0.4% decrease; $P = 0.608$) in the prevalence of single drug resistance between 1999 and 2000 and 2000–2001. There were significant changes in the prevalences of pan-susceptibility (3.0% increase; $P = 0.002$) and 2 drug resistance (DR) (2.2% decrease; $P < 0.001$). MDR was present in 618 (10.8%) SP collected during 2001–2002 and 552 (11.1%) SP collected in 1999–2000 (0.4% decrease; $P = 0.523$). LEV R was present in 4.7% of the MDR isolates compared with 4.2% in 1999–2000 ($P = 0.764$). AZI R as a component of MDR increased between 1999 and 2000 (90.3%) and 2001–2002 (94.6%), although not significantly ($P = 0.599$). TMP-SMX R as a component of MDR decreased 1.1% ($P = 0.400$) between 1999 and 2000 (100%) and 2001–2002 (98.9%). By country, the prevalence of MDR SP ranged from 0.4% in Germany to 50.9% in South Korea. The largest increase in MDR (23.2%) from 1999 to 2000 occurred in Hong Kong, due to an increase in AZI R. In Thailand, the prevalence of 2 DR and MDR both showed an increase (2.7 and 8.5%, respectively), also attributed to increased AZI R. South Korea showed a decrease in both 2 DR and MDR (4.3 and 7.2%, respectively) due to a decrease in TMP-SMX R.
Conclusion: Despite an increase in the number of pan-susceptible isolates, MDR continued to be a significant problem in some countries, exceeding 20%. LEV susceptibility, as a marker for other respiratory fluoroquinolones, remained high and least associated with MDR SP compared with other antimicrobial classes.

P1252 Correlations in antimicrobial resistance among *Streptococcus pneumoniae* and *Haemophilus influenzae*: 2001–2002 international surveillance

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Objectives: Antimicrobial resistance (R) rates to commonly prescribed agents such as beta-lactams, macrolides and trimethoprim-sulfamethoxazole (TMP-SMX) for *Streptococcus pneumoniae* (SP) and *H. influenzae* (HI) can vary by region. We analyzed correlations in R among SP and HI from different countries to determine if R rates to selected agents varied in unison for the two species.
Methods: During 2001–2002, 5926 SP and 5319 HI were collected from patient specimens at 80 hospital laboratories in 13 countries in Africa, Asia, Europe, and North and South America. Isolates were centrally tested by broth microdilution against penicillin (PEN; SP only), ampicillin (AMP; HI only), amoxicillin-clavulanate (AC), cefuroxime (FUR), clarithromycin (CLR), levofloxacin (LEV), and TMP-SMX. MICs were interpreted using 2002 NCCLS breakpoints. Relationships between SP and HI to individual agents between countries were tested by measuring the correlation coefficient (r) of percent R in each species using a t-test analysis.
Results: R to beta-lactams, CLR and TMP-SMX among SP and HI varied considerably between countries, with the highest R rates generally being reported in Asia. LEV R among SP was $\leq 1.5\%$ in all countries except South Korea (2.1%) and Hong Kong (8.5%) and was undetected in HI. Statistical tests showed a highly significant relationship ($r = 0.886$; $P < 0.001$) between PEN R in SP and AMP R in HI. Similarly, a significant relationship was observed for TMP-SMX R for these species between countries ($r = 0.609$; $P = 0.05$). No significant correlation was detected for FUR or CLR. Correlations in R rates to AC or LEV among SP and HI could not be determined due to low R (0% in many countries) to the two agents among HI. However, a high incidence of AC R among SP (16.6%) and HI (1.2%) was recorded in South Korea.
Conclusions: Beta-lactam R and TMP-SMX R in SP and HI arise from separate mechanisms in each species. Thus, the correlations in R between the species may indicate a response to a common selective pressure, most likely antimicrobial consumption. Examination of antimicrobial consumption coupled with annual surveillance will provide further understanding of these relationships.

P1253 A longitudinal assessment of antimicrobial resistance among *Haemophilus influenzae* and *Moraxella catarrhalis* collected worldwide during 1999–2002

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Objectives: *Haemophilus influenzae* (HI) and *Moraxella catarrhalis* (MC) are common respiratory pathogens worldwide. In some countries, high rates of beta-lactamase (BL) production and resistance (*R*) to trimethoprim-sulfamethoxazole (TMP-SMX) have challenged the empiric treatment of HI and MC. Fluoroquinolones such as levofloxacin (LEV) have provided an alternative therapy for these infections. Currently, LEV R is extremely rare, however, the continued monitoring of R rates is necessary to track any changes in R, should they occur.
Methods: During 1999–2000 and 2001–2002, HI (1999–2000, $n = 4640$; 2001–2002, $n = 5138$) and MC (1999–2000, $n = 556$; 2001–2002, $n = 1014$) were collected from patient specimens in hospital laboratories in 12 countries. Isolates were tested by NCCLS broth microdilution against ampicillin (AMP), azithromycin (AZI), LEV, TMP-SMX, and comparator agents. MICs were interpreted using the 2002 NCCLS published breakpoints.
Results: Overall, BL production in HI decreased 1.5% between 1999 and 2000 (19.0%) and 2001–2002 (17.5%). This decrease was accompanied by a 0.9% decrease in AMP R (1999–2000, 18.4%; 2001–2002, 17.5%). The largest increase in BL production (7.3%) and AMP R (7.1%) was detected in China. Seven AZI nonsusceptible (NS) HI (Germany, 1; Italy, 1; South Africa, 2; Spain, 3) were collected in 2001–2002 compared with none in 1999–2000. Overall TMP-SMX R decreased 4.6% from 1999 to 2000 (28.2%) to 2001–2002 (23.6%). Mexico showed the largest decrease (10.8%) in TMP-SMX R between 1999 and 2000 (35.8%) and 2001–2002 (25.0%). The largest increase (12.3%) was detected in South Korea (1999–2000, 44.4%; 2001–2002, 56.7%). No LEV NS HI were collected in either year. Overall, BL production among MC increased 3.1% from 93.5% in 1999-2000, 96.6% in 2001–2002. MIC90s for AMP and AZI increased one doubling dilution from 4 to 8 mg/L and ≤ 0.03–0.06 mg/L, respectively, between 1999 and 2000 and 2001–2002. Based on MIC90, LEV (0.06 mg/L) was the most active agent tested against MC in 2001–2002, unchanged since 1999–2000.
Conclusions: The high prevalence of beta-lactam R and TMP-SMX R among HI and MC in some countries highlights the need for alternative therapies such as LEV to treat respiratory infections. Continued surveillance on a worldwide scale will provide insights into changing dynamics of antimicrobial R among HI and MC.

Skin and soft tissue

P1254 Panton-Valentine leukocidin associated epidemic of soft tissue infections in the San Francisco community

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Objective: To describe the prevalence of Panton-Valentine leukocidin (PVL) producing *S. aureus* strains in patients with nosocomially and community-acquired infections in the Community Health Network of San Francisco (CHN), to identify the genotypes of these isolates and determine the Staphylococcal Chromosomal Cassette (SCC) mec type conferring their resistance to methicillin.
Methods: The PVL -carrying genes (lukS-PV and lukF-PV) and SSCmec types were determined by PCR. Genotyping consisted of pulsed field gel electrophoresis following SmaI digestion (PFGE) and multilocus restriction fragment typing (MLRFT).
Results: In a survey of 197 *S aureus* strains recovered from patients in the CHN between August 1999 and April 2000, we identified 32 (16.2%) isolates that carried the lukS-PV and lukF-PV genes. As defined by PFGE and MLRFT, 27 (84.4%) of these belonged to a single clonal lineage: SF13. Twenty-four (89%) of the 27 PVL + SF13 isolates also carried the SCC mec typeIV. Moreover, PVL + isolates were more likely to have been recovered from abscess/wound sites than sterile sites (37.5 and 6%, $P = <0.0001$). To assess the geographic spread of PVL + SF13 isolates, we examined 47 random MRSA collected between January 2000 and July 2001 from a public adjacent county. Fourteen (29.8%) of them carried the PVL genes; of these, 10 (71.4%) belonged to the SF13 clonal lineage and carried SCC mec type IV. In view of the increased prevalence of community-acquired soft tissue infections in San Francisco, we analyzed 65 consecutive MRSA isolates collected between July 1 and October 15, 2000 from abscess/wound cultures, in a prospective study in patients receiving surgical treatment at the Integrated Soft-tissue Infections Services at San Francisco General Hospital (as part of CHN). Of these, 44 (67.7%) carried the PVL genes and 34 of the 44 (77.3%) belonged to the SF13 clonal lineage and carried the SCC mec type i.v. element.
Conclusion: A Panton-Valentine leukocidin-associated epidemic of soft-tissue infections is due to a single methicillin-resistant *S. aureus* clone carrying the SCC mec type i.v. element in the San Francisco community.

P1255 Community-acquired cutaneous infections due to different methicillin-resistant *Staphylococcus aureus* strains

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Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections have recently been reported in patients from different countries, without known risk factors for acquisition of nosocomial bacteria. Some strains present specific virulence factors, as the Panton-Valentine toxin that is associated with skin infections and more rarely necrotizing pneumonia with a high mortality. Our purpose was to characterize CA-MRSA identified by our laboratory during the year 2002. From January to December 2002, 12 isolates of CA-MRSA have been isolated from 10 patients with cutaneous infections. None of them was at risk to acquired nosocomial infections. There were six patients, four children and two adults from three different families, who presented furonculosis. Three other children presented non bullous impetigo and one adult a folliculitis. Identification of *Staphylococcus aureus* was obtained by a positive coagulase tube test. All the isolates were resistant to methicillin, confirmed by the presence of the mecA gene. Isolates were also resistant to kanamycin ($n = 9$), fusidic acid ($n = 10$), tetracyclines ($n = 12$), erythromycin ($n = 3$) and cotrimoxazole ($n = 2$). Toxinotyping was performed by PCR. Pulsed Field Gel Electrophoresis (PFGE) of genomic DNA was made after digestion by SmaI. All the isolates had the LukE-Lu kDa leukotoxin genes. The eight isolates obtained from patients with furonculosis had the Panton-Valentine toxin genes, an *agr3* allele and showed the same pattern by PFGE. From the three patients with impetigo, two have isolates characterized by the presence of exfoliative A toxin gene, an *agr3* allele and a similar PFGE pattern. The third strain isolated from a patient with impetigo had the Panton-Valentine toxin genes, enterotoxin (enterotoxins M and O) genes and an *agr2* allele. The isolate from folliculitis did not have Panton-Valentine toxin neither exfoliative toxin genes and had an *agr3* allele. These results emphasize the emergence of community-acquired cutaneous infections due to different strains of CA-MRSA. MRSA Panton-Valentine producing isolates linked to furonculosis represent an emerging clonal group, which is the same that the one described recently in France. MRSA exfoliative A producing isolates represent a different clone. Both practitioners and microbiologists should be aware of this new type of MRSA infections.

P1256 Community-acquired methicillin-resistant *Staphylococcus aureus* in Greek children

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Objective: Historically, infections due to MRSA have been considered a nosocomial problem. More recently, serious MRSA infections have been acquired in the community. We aimed to define the frequency of MRSA in pediatric patients of a tertiary care hospital and estimate the proportion of community-acquired MRSA (CA-MRSA).
Methods: All cases of pediatric patients from whom *S. aureus* including MRSA was isolated between January 1999 and December 2001 were reviewed. CA-MRSA was defined as a MRSA isolated from a culture obtained at or within 2 days after hospital admission from a patient not hospitalized during the previous 6 months. Chi square test was used for comparison of proportions.
Results: *S. aureus* was isolated from 123 children and MRSA was responsible for 45 (36.6%) cases. In 19 of these 45 patients (42.2%), MRSA was nasal colonizer. The children with MRSA were aged 1 d to 17 year. In general, MRSA tended to be isolated from boys and infants younger than 45 d more frequently than girls and older children, respectively ($P = 0.07$) (Table). Trends of differences among the years are not significant. Forty-one MRSA isolates (91.1%) were susceptible to clindamycin.

Year	*S. aureus*	MRSA	CA-MRSA
1999	39	15 (38.5%)	5/15 (33.5%)
2000	49	21 (42.9%)	8/21 (38.1%)
2001	35	9 (25.7%)	4/9 (44.4%)
Total	123	45 (36.5%)	17/45 (37.8%)

Conclusions: (i) More than one-third of MRSA's isolated from pediatric patients in our hospital are community-acquired. Frequencies of both MRSA and CA-MRSA tend to remain constant through the study period. (ii) Clindamycin is active against almost all MRSA strains and thus valuable for empirical treatment of serious staphylococcal infections. (iii) Control of MRSA requires surveillance for MRSA and appropriate use of antibiotics not only in the hospital but also in the community.

P1257 A community outbreak of methicillin-resistant *Staphylococcus aureus*

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Objectives: In Norway, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) has been increasing from 21 in 1995 to 122 in 2001. MRSA has hereto largely been confined to hospitals and long-term care facilities, and known risk factors have been prior antibiotic usage, admission to ICU, surgery and exposure to other MRSA-colonized patients. However, during the last years the relative frequency of community-acquired MRSA has increased. In 2001, 57% of the 122 registered MRSA in Norway were from patients outside hospital. It is not known whether these community-acquired isolates are bacteria escaping and spreading from hospitals, or if this isolates have arisen as a consequence of horizontal transfer of genes conferring methicillin-resistance into a formerly susceptible *S. aureus* population. In a small urban community in Telemark, Norway, there has been an outbreak with MRSA including eight patients during a 15-month period starting in July 2001 and ending in October 2002. All patients presented with abscesses.

Methods: Susceptibility testing, pulsed-field gel electrophoresis, patient screening and interview.
Results: The eight isolates of MRSA were only resistant to beta-lactam antibiotics and fusidic acid, and showed identical PFGE patterns. By comparison with PFGE patterns of strains in the Swedish MRSA database, this Norwegian outbreak strain was found to be indistinguishable from the strain DK E97-1, a strain that was first isolated and characterized in Denmark, but which origin is in the Eastern Mediterranean area. It was found that the first patient had been treated at a Greek hospital during a holiday prior to the identification of MRSA in July 2001. The following seven patients were relatives or close social contacts.
Conclusion: Norway has guidelines for preventing MRSA from entering hospitals which includes screening of patients and health care workers from endemic areas. Guidelines should also include recommendations for how to take care of patients with MRSA outside hospitals.

P1258 Nontyphoidal salmonella abscess in an immunocompetent adult: case report

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The clinical spectrum of extra-intestinal salmonella infection includes enteric fever (typhoid and paratyphoid) and invasive infections due to nontyphoidal salmonellae, such as osteomyelitis, septic arthritis, meningitis, arterial infections and bacteremia. A number of host factors can predispose to extra-intestinal *Salmonella* infections, although a number of cases in otherwise healthy individuals have been reported. A 58-year-old-man was admitted to Henry Dunant Hospital for a 3-day history of fever, malaise and a painful swelling of the right parotid. For 2 days before his admission he had being taken amoxicillin and metronidazole as this was thought to be related to dental infection, by his general practitioner. As the symptoms did not seem to withdraw, the patient was referred to the hospital's ENT department for evaluation. His white blood count was 9250/mm^3 (69.7% neutrophils, 17.2% lymphocytes and 6.8% monocytes), hematocrit 48.5%, hemoglobin level 16.1 g/dL, platelet count 267 000/mm^3, C-reactive protein 8.3 mg/dL and erythrocyte sedimentation rate 70 mmHg. No evidence of palpable lymph nodes was noted and the chest X-ray film showed no abnormalities. CT scan revealed an abscess of the right parotid, with no evidence of malignancy. The patient did not suffer from any immune or endocrine disease, nor taken any other medication in the past. He did not remember to suffer from diarrhea or any other syndrome for the last months. The patient underwent fine-needle aspiration of the abscess under U/S guidance and the pus aspirated was sent to our microbiology laboratory for culture. After 24-h incubation, *Salmonella enteritidis* was isolated from the sample. The stool, urine and blood cultures were negative. The patient was treated with ciprofloxacin 400 mg p.o. q12 h for 15 days, with complete remission of symptoms. He was revised 20 days later and he showed complete resolution of the infection. The last decade, *Salmonella enteritidis* is the most frequent of nontyphoidal Salmonellae causing extra-intestinal infections. However, focal soft tissue infections due to Salmonellae are still rare and diverse underlying conditions are present in the majority of patients. In our case, the patient was an otherwise completely immunocompetent individual, with no risk factors predisposing to this infection.

P1259 Recurrent intradermal abscesses caused by *Citrobacter freundii* in an immunocompetent host

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Objective: *Citrobacter* species in the family Enterobacteriaceae are infrequent human pathogens associated with urinary tract infections and bacteremias in hospitalized or immunocompromised patients, as well as with sepsis, meningitis and brain abscesses in newborns. *C. freundii* has also been reported to cause endocarditis, spondylodiscitis and toxic gastroenteritis in healthy hosts. Here we report on a healthy young woman with recurrent intradermal abscesses following a cat scratch.
Methods: A 20-year-old woman was hospitalized because of the 5th recurrence of a intradermal abscess of her left forearm. Abscesses had appeared lined in close sequence towards the arm root, each a month after the other, with fever, malaise, tenderness and swelling of the whole arm. In spite of short courses of oral amoxicillin/clavulanate, they resolved only after surgical drainage. Blood and pus samples were cultured on admission, and i.v. ceftriaxone and ciprofloxacin administered for 2 weeks with apparent resolution. Three weeks later she came back with another intradermal abscess on her left flank. Clindamycin and ciprofloxacin were started i.v. after blood and pus sampling. Culture isolates were identified with an automated VIDEK system (bioMerieux) and stored for strain collection.
Results: Pus cultures grew *C. freundii* as the only isolate on both occasions, blood cultures remaining sterile. Isolates from both abscesses shared resistance to amox/clav, whereas those from the 2nd one showed decreased sensitivity to ciprofloxacin. Thorough immunologic workup of the patient failed to reveal any significant immune deficit. Histology of lesions was unremarkable.
Conclusions: To our knowledge this is the first report of intradermal abscesses caused by *C. freundii* in a immunocompetent host, likely infected through an unusual pathway. Infection was hard to control, as indicated by recurrence by a selected ciprofloxacin-resistant strain, adding to the knowledge that this unusual pathogen may represent a relevant therapeutic challenge.

P1260 Multiple splenic abscesses due to *Bartonella henselae*

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Introduction: Cat scratch disease (CSD) is usually characterized by a self-limited regional lymphadenopathy but systemic manifestations has been described, with ocular, neurological and visceral organ involvement. Less recognized is the picture of multiple splenic abscesses, whose differentiation from pyogenic abscesses, infarctions, lymphomas and systemic diseases could be difficult. A previously healthy 37-year-old patient was admitted with a 14-day history of spiking fever to 40.0°C with chills and epigastric pain. On admission he was febrile at 39.7°C and showed normal hemodynamic parameters. Physical examination was unremarkable except for a weak epigastric pain. Laboratory investigations showed slight inflammatory response and elevated liver enzymes. A CT scan of the abdomen revealed a hepatomegaly, enlarged portal lymph nodes, and multiple hypodense lesions up to 2.5 cm in the spleen. Broad spectrum antibiotic therapy was started with Imipenem (1 g i.v. tid). Clarithromicin was added (500 mg i.v. bid) because the history of a cat scratch raised the suspicion of a *Bartonella* infection. Multiple blood cultures were negative and a transesophageal echocardiography was unremarkable. The fever disappeared after 10 days of treatment. A serology confirmed the diagnosis of *Bartonella henselae* infection with a IgG titer of 1 : 4096 ($n < 50$)
Discussion: Multiple splenic hypodense lesions in a high febrile patient require both extensive diagnostic work-up and rapid therapeutic action. The clinical presentation in our patient was not suggestive for a neoplastic disease. In the immunologically competent host, splenic abscesses are uncommon and frequently the consequence of bacteremic infections (mostly endocarditis but also urinary, abdominal or respiratory tract infections). Most are caused by aerobic or anerobic bacteria but very rarely unusual pathogens such as fungi or mycobacteria are involved. Atypical presentations of *B. henselae* infections include the so-called hepatosplenic CSD. The radiologic aspect ranges from diffuse small granulomas in the liver and/or spleen to larger abscesses with or without lymphadenopathies. A high index of suspicious and a prompt diagnosis is required to avoid a splenectomy, which is usually indicated if splenic abscesses are due to pyogenic pathogens.

P1261 Natural history and complications of deep venous thrombosis in intravenous drug users

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Objectives: We hoped to document the nature of infective complications in i.v. drug users (IVDU) with deep venous thrombosis (DVT).
Methods: A case note audit of all DVT admissions in adults under 40 years of age over a 5-years. A questionnaire was used to extract data concerning i.v. drug use, investigation, nature and complications of DVT. In addition we looked at all previous an subsequent DVTs in these patients.
Results: (see Tables 1–3).

Table 1

Risk factor	Number total=181
IVDU	98 (54.1%)
Smoker	131(72.4%)
Previous DVT	50 (27.6%)
Family history	18 (9.9%)
Thrombophilia	19 (10.4%)
Recent surgery	9 (5.0%)
Other immobility	18 (9.9%)
Cancer	3 (1.65%)
Female contraceptive	6 of 72 (8.3%)
SLE	0
Chronic inflammation	16 (8.8%)
Obesity	2 (1.1%)
Steroid	3 (1.65%)
Alcoholism	27 (14.9%)

Table 2

Result of blood culture	Number of patients with possible DVT (in brackets)
Staphylococcus aureus	23 (17)
Enterococcus faecalis	2 (2)
Enterococcus faecalis and *Staphylococcus aureus*	1 (0)
Moraxella oslensis	3 (2)
Streptococcus viridans	2 (2)
Group B *Streptococcus*	2 (0)
Group B *Streptococcus* and *Staphylococcus aureus*	1 (1)
Group A *Streptococcus*, Group B *Streptococcus* and *Staphylococcus aureus*	1 (0)
Group A *Streptococcus*	1 (1)
Group C *Streptococcus*	1 (1)
Group A *Streptococcus*, Group B *Streptococcus* and *Staphylococcus aureus*	1(1)
Staphylococcus aureus and *Streptococcus oralis*	1 (1)
Streptococcus milleri and a Gram negative rod	1 (1)
Streptococcus milleri	1 (1)
Proteus mirabilis	1 (1)
Total	43 (33)

There were 98 IVDU (all groin injectors) and 83 non-IVDU patients admitted with proven DVT during the study period. From the case notes we were able to document 134 additional admissions for possible DVT in IVDUs and 17 for non-IVDUs. IVDUs had longer hospital stays with proven or possible DVTs. IVDUs were more likely to be an inpatient on the ID Unit ($P<0.001$). For all admissions, IVDUs had a high rate of self discharge. The majority of IVDU patients had proximal DVT (16.9% of non-IVDUs). There was no difference in the incidence of pulmonary embolism between the two groups but septic pulmonary emboli were common in IVDU patients. Infective complications rare in non-IVDU but occurred in nearly half of IVDUs. Of a total of 232 admissions with DVT in i.v. drug users, blood cultures were taken on 101 occasions. There were 43 positive cultures, of which 33 occurred in patients with confirmed DVT. There were no known HIV positive patients. IVDUs needed significantly more additional admissions to exclude a possible DVT. The risk of recurrence in the 3 and 6-month period after discharge was significantly higher for IVDUs ($P<0.001$). The number of days until recurrence was not associated with the length of time anticoagulated (Pearson correlation). IVDUs seen on the ID Unit had a longer hospital stay (MW, $P=0.028$), were less likely to be offered warfarin therapy (Chi, $P=0.002$) and received longer anticoagulation (MW, $P=0.039$). Patients admitted to the ID unit were more likely to have had blood cultures taken. Interpretation: This study confirms the importance of i.v. drug use as a risk factor for DVT of the lower limb (54.1% associated with IVDU). We describe DVT in i.v. drug users as a significantly different disease process commonly presenting to Infectious Diseases physicians. Treatment regimes differ between medical wards. Such patients commonly presenting with septic complications, require particular treatment and pose distinct problems regarding their hospital stay and follow-up.

Table 3

	IVDU ($n=98$)	Non-IVDU ($n=83$)	
Type of DVT			
Proximal	66 (67.3%)	14 (16.9%)	$P<0.001$
Distal	32 (32.7%)	69 (83.9%)	
Associated problems			
Cellulitis	47 (48.2%)	2 (2.4%)	$P<0.001$
Other STI	46 (46.9%)	1 (1.2%)	$P<0.001$
Septacemia	24 (24.5%)	0	$P<0.001$
PE	9 (9.2%)	7 (8.4%)	NS
Septic emboli	15 (15.3%)	0	$P<0.001$

Epidemiology of resistance 4

P1262 Telithromycin is highly active against *Streptococcus pneumoniae* isolates, including resistant strains, from pediatric patients: PROTEKT Year 1 vs. Year 2

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Background: Treatment of *Streptococcus pneumoniae* infections in pediatric patients is often complicated by high local prevalences of resistance to beta-lactams and macrolides. As part of the PROTEKT surveillance study, the activity of the first ketolide telithromycin (TEL) was investigated against *S. pneumoniae* isolated from pediatric patients in 1999–2000 (Y1) and 2000–2001 (Y2).
Methods: *S. pneumoniae* (Y1, $n=802$; Y2, $n=1164$) were isolated from children aged $\leq$14 years with community-acquired RTIs in $\geq$20 countries worldwide. Isolates were tested against a panel of antibacterials, including penicillin (PEN), erythromycin (ERY) and TEL, according to NCCLS guidelines. Macrolide resistance mechanisms were determined, using PCR, in ERY-R (MIC $\geq$ 1 mg/L) isolates.
Results: The prevalence of PEN-I (MIC 0.12–1 mg/L), PEN-R (MIC $\geq$ 2 mg/L) and ERY-R varied globally but was particularly high in several countries, notably Japan and South Korea in both years (Table).

	1999–2000 (Y1)				2001–01 (Y2)			
		% of isolates				% of isolates		
Country*	Total no. of isolates	PEN-I	PEN-R	ERY-R	Total no. of isolates	PEN-I	PEN-R	ERY-R
France	28	21	46	71	11	27	36	55
Hungary	24	46	17	63	45	9	31	31
Japan	114	21	51	81	291	23	40	79
S. Korea	31	10	81	97	34	24	71	94
Worldwide	802	18	25	36	1164	15	23	40

*Countries with high prevalence of resistance and >10 *S. pneumoniae* isolates.

Preliminary genotype analysis indicates similar prevalence of macrolide resistance genes among ERY-R isolates for both years: Y1: *erm(B)* 51%, *mef(A)* 38%, *erm(B)* + *mef(A)* 11%; Y2: *erm(B)* 61%, *mef(A)* 35%, *erm(B)* + *mef(A)* 4%. TEL (NCCLS proposed susceptibility breakpoint $\leq$1 mg/L) inhibited 100% of isolates (MIC90 0.25 mg/L) in Y1 and 1162 (99.8%) of isolates (MIC90 0.12 mg/L) in Y2.
Conclusions: These data confirm the continuing high worldwide prevalence of PEN and ERY resistance among *S. pneumoniae* isolated from pediatric patients in several countries. Telithromycin exhibits excellent activity against *S. pneumoniae* from pediatric patients, including PEN-I, PEN-R and ERY-R isolates.

P1263 High prevalence of antibacterial resistance among *Streptococcus pneumoniae* collected from patients with community-acquired respiratory tract infections in Japan: three years of PROTEKT data

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Objectives: Japan has high levels of antibacterial resistance, including multidrug resistance, among *S. pneumoniae*. PROTEKT provides annual surveillance of resistance among community-acquired respiratory tract infections (RTIs) pathogens. International antimicrobial surveillance studies play a critical role in defining the nature and extent of this problem as well as in guiding prescribing.
Methods: *S. pneumoniae* were collected from Japanese patients with community-acquired RTIs during the 1999/00 (Y1), 2000/01 (Y2) and 2001/02 (Y3) seasons. Susceptibilities (NCCLS methodology) and macrolide resistance genotypes were determined at a central laboratory.
Results: Centres in Japan submitted a total of 308, 627 and 816 pneumococcal isolates in Y1, Y2 and Y3, respectively. There was no marked change in the MIC90 of the antibacterial agents over the three years. The frequencies of the resistant isolates of *S. pneumoniae* to the major antibiotics are shown in Table 1. High levels of resistance to penicillin, erythromycin, clindamycin and tetracycline continued over the 3 years. Resistance to fluoroquinolones has remained at a low level (despite high levels in neighboring Hong Kong). The MIC90 of telithromycin has stayed low over the 3 years (0.25, 0.12, 0.12 mg/L, respectively), and >99.5% of isolates each year were susceptible to telithromycin at ≤1 mg/L. Over the 3 years, erm(B)-(Y1 52.7, Y2 63.4, Y3 59.4%) continued to be more prevalent than mef(A)-mediated (Y1 42.7, Y2 34.1, Y3 37.0%) macrolide resistance. The prevalence of isolates with mef(A) + erm(B) remained low (Y1 3.4, Y2 1.9, Y3 3.4%).

Table 1

	Resistant isolates (%)		
Antibacterial agent	Y1	Y2	Y3[a]
Penicillin[b]	64.3	54.8	64.0
Erythromycin	77.9	77.2	79.9
Clindamycin	44.8	50.6	49.4
Levofloxacin	1.3	1.1	1.1
Tetracycline	78.6	79.9	83.6
Co-trimoxazole	10.7	8.1	7.4

[a]Provisional data.
[b]intermediate + resistant (MIC ≥ 0.12 mg/L).

Conclusion: Japan, in common with other Asian countries, has high levels of antimicrobial resistance to many first-line antibiotics. Telithromycin, the first ketolide antibiotic, exhibits high potency against *S. pneumoniae* including multiresistant strains.

P1264 Demography and antimicrobial susceptibility of community-acquired respiratory tract infection pathogens in Year 2 vs. Year 1 of the PROTEKT Surveillance Program

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Objectives: PROTEKT is a global longitudinal surveillance study of antimicrobial susceptibility among community-acquired respiratory tract infections (CARTI) pathogens. Data from the first 2 years (Y1: 1999–2000; Y2: 2000–01) are presented.
Methods: Isolates of *S. pneumoniae* (SPN), *H. influenzae* (HI), M. catarrhalis (MC), *S. pyogenes* (SPY) and *S. aureus* (SA) from patients with CARTIs in 25 (Y1) and 22 (Y2) countries were tested at a central laboratory for antimicrobial susceptibility (NCCLS).

Table 1

	% of isolates for which demographic data were provided[a]									
	Y1					Y2				
Demography	SPN, $n=3362$	HI, $n=2948$	MC, $n=1131$	SPY, $n=1485$	SA, $n=1547$	SPN, $n=4256$	HI, $n=3133$	MC, $n=1156$	SPY, $n=1364$	SA, $n=1493$
Age group										
0–14	24	23	28	46	13	27	26	28	52	14
15–64	36	35	33	32	46	37	36	31	33	47
≥65	25	24	30	4	24	26	28	33	4	31
Source										
Sputum	44	54	60	5	32	42	55	59	4	37
Blood	15	2	2	3	13	16	1	1	3	18
NAP	7	9	12	3	8	11	11	13	4	11
BAL	8	9	8	1	10	9	10	7	1	10
Other	13	10	10	71[b]	21	14	14	12	85[b]	18

NAP: nasopharygeal swab/aspirate BAL: bronchoalveolar lavage, [a]data not available for all isolates, [b]mainly troat swabs

Results: Demographic data are shown in Table 1.
Rates of resistance to penicillin (PEN-R) and erythromycin (ERY-R) in SPN were similar across the three age groups in both years (PEN-R: Y1, 19–25%, Y2 15–23%; ERY-R: Y1 29–36%, Y2 29–40%). Overall, PEN-R and ERY-R in both years were highest in the Far East (Y1: $n=515$; 53 and 80%; Y2: $n=718$; 34 and 77%, respectively) and France (Y1: $n=184$; 46 and 58%; Y2: $n=191$; 39 and 54%, respectively). Beta-lactamase (BL) production in HI was highest in South Korea (Y1: 33/51, 65%; Y2: 31/55, 56%). Among MC, BL production was >90% in most countries for both years. Worldwide, ERY-R remained constant for SPY (Y1 9%; Y2 10%) and for methicillin-susceptible SA (MSSA; Y1: 246/1239, 20%; Y2: 219/1183, 19%). Telithromycin (TEL) was highly active against all pathogens, with the same MIC90 values for both years for SPN 0.12 mg/L, HI 2 mg/L, MC 0.12 mg/L and SPY 0.015 mg/L; MIC90 for MSSA were: Y1 0.12 mg/L, Y2 0.06 mg/L. At ≤1 mg/L in both years, TEL inhibited 99.9, ≥97.6 and ≥95.3% of SPN, SPY and MSSA, respectively. At ≤4 mg/L, telithromycin inhibited ≥99.6% of HI and 100% of MC in both years. TEL susceptibilities were not markedly affected by patient demography or resistance to other antimicrobial agents.
Conclusion: Antimicrobial resistance continues to be a global problem. TEL, the first ketolide to be approved for clinical use, has excellent in vitro activity against the CARTI pathogens studied, including antimicrobial-resistant SPN, regardless of demographic factors.

P1265 Hot spots of antibacterial resistance among community-acquired respiratory tract infection pathogens in Europe: 2 years of data from the PROTEKT study

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Objectives: Patterns of antibacterial resistance among community-acquired respiratory tract infections (CARTIs) pathogens vary between European countries. In some countries, the prevalence of resistance to penicillins and macrolides approaches the high levels seen in the Far East. PROTEKT is a global surveillance study monitoring antibacterial resistance among CARTI pathogens. We report here the major changes in resistance patterns observed in European countries during the first 2 years of PROTEKT.
Methods: Clinical isolates of *S. pneumoniae* (SPN), *Haemophilus influenzae* (HI) and *Moraxella catarrhalis* (MC) from respiratory samples were submitted from European centers during the winters of 1999/2000 (Y1) and 2000/2001 (Y2). MICs and susceptibilities were determined according to NCCLS guidelines.
Results: In Y1 and Y2 a total of 1521 and 2331 SPN, 1288 and 1609 HI, and 527 and 619 MC were collected, respectively. Increases in resistance rates were seen in Portugal (penicillin, cotrimoxazole) and Hungary (penicillin, cotrimoxazole). Conversely, falls in resistance rates were seen in Poland and Eire (penicillin, erythromycin, cotrimoxazole, tetracycline), in Spain (penicillin) and Hungary (erythromycin, tetracycline). Beta-lactamase production in HI fell in Spain from 26.8 to 10.9%, and rose in Ireland from 7.9 to 26.0%. MC beta-lactamase production was >80% in all countries. In Y1 and Y2, telithromycin at ≤1 mg/L inhibited >99.5% of SPN, >99.5% of HI and 100% of MC.

	Resistance (%) among isolates of SPN									
	n		Penicillin		Erythromycin		CO-trimoxazole		Tetracycline	
	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2	Y1	Y2
France	184	191	46.2	38.7	57.6	53.9	43.1	30.4	44.0	47.1
Germany	325	693	2.2	2.4	15.7	15.9	8.3	7.4	11.7	14.0
Hungary	54	60	13.0	33.3	55.6	31.7	44.4	53.3	63.0	40.0
Ireland	53	57	34.0	26.3	26.4	17.5	37.7	24.6	30.2	10.5
Italy	119	284	10.0	6.3	42.9	34.9	23.5	26.8	25.2	21.5
Poland	68	77	13.2	6.5	23.5	2.6	26.5	9.1	45.6	18.2
Portugal	106	93	10.4	26.9	16.0	12.9	21.7	35.5	19.8	14.0
Spain	133	422	42.1	30.1	28.6	35.3	48.9	46.4	36.1	37.6
Turkey	77	101	14.3	11.9	15.6	13.9	35.1	31.7	24.7	19.8

Conclusion: Resistance to several first line antibacterials is a continuing problem in Europe. The first 2 years of PROTEKT indicate changing patterns of resistance to several antibacterial agents in individual countries. Telithromycin exhibited excellent in vitro activity against the principal CARTI pathogens, including strains resistant to other agents.

P1266 Antimicrobial resistance surveillance of *Staphylococcus aureus* isolates in a pediatric population (1998–2002)

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Objectives: To study the incidence of methicillin resistant *Staphylococcus aureus* (MRSA), the main resistance phenotypes and to identify trends in antimicrobial resistance throughout a 5-year period (1998–2002).
Methods: We retrospectively analyzed the antimicrobial susceptibility of 1796 nonduplicate *S. aureus* isolates, recovered from various clinical specimens (infection or colonization sites), from children 0–14 years old, treated at a Tertiary Care Children's Hospital in Athens. The standard disk diffusion method was used routinely to determine antimicrobial resistance (NCCLS recommendations). The Penicillin Binding Protein Latex Agglutination test (OXOID) was used to evaluate marginal resistance to methicillin or to obtain a quick result in serious infections.
Results: Overall, 93.4% of the isolates were resistant to penicillin (PEN), 16.9% to oxacillin (OXA), 4.3% to gentamicin (GEN), 5.0% to tobramycin (TOB), 11.3% to kanamycin (KAN), 13.6% to tetracycline (TET), 16.6% to erythromycin (ERY), 2.7% to clindamycin (CLI) and 1.6% to cotrimoxazole, while all were susceptible to glycopeptides. Annual oxacillin resistance rates of 18.8, 10.6, 18.4, 15.7 and 20.3% were noted for the years 1998–2002, respectively. The incidence of resistance was significantly higher among the oxacillin resistant isolates than the sensitive ones for GEN (15.8 vs. 1.9%), TOB (17.8 vs. 2.4%), KAN (45.0 vs. 4.1%), TET (29.0 vs. 10.1%), ERY (20.1 vs. 15.4%) and CLI (9.3 vs. 1.4%). The main resistance patterns were PEN (1316), PEN-OXA (179), PEN-OXA-GEN-TOB-KAN (40), PEN-GEN-TOB-KAN (26), PEN-OXA-KAN (43) and PEN-KAN (13). The incidence of oxacillin resistance was relatively higher among the isolates from blood (20.4%), from surgical specimens (18.8%), orthopedic specimens (18.0%) and from Special Care Units (20.7%) than the isolates from Nephrology Unit patients (11.1%) and children treated as outpatients or admitted in other Pediatric Wards (14.2%).
Conclusions: Methicillin resistance was found in about one-fifth of pediatric *S. aureus* isolates, with no steadily increasing or decreasing trends during the recent years. MRSA isolates were more frequently concomitantly resistant to other antimicrobials than methicillin sensitive ones. The monitoring of *S. aureus* resistance is required to help in therapeutic decisions for empirical therapy, which must be accompanied by appropriate cultures for isolation and antimicrobial testing of the *S. aureus* strains.

P1267 Antimicrobial resistance of pathogens isolated from aural infections in a Greek pediatric hospital

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Objectives: This study records and analyzes the pathogens recovered from ear fluid cultures and their susceptibility to antibiotics during the period 2000–2001.
Methods: A total of 1764 samples of otic exudates from children aged 23 days to 14 years were studied. The subjects were either examined at the outpatient department of 'P. & A. Kyriakou' Children's Hospital or were admitted for hospitalization. The antibiotic susceptibility of the isolated pathogens was determined by the disk diffusion method and the MIC of *Streptococcus pneumoniae* strains to selected β-lactams was determined by the *E*-test method according to the NCCLS guidelines.
Results: A total of 1288 cultures were positive. More than one pathogen was isolated from 131 cultures. 1481 isolates were examined; these included 319 *Streptococcus pneumoniae* isolates, 305 *Pseudomonas aeruginosa*, 297 *Staphylococcus aureus*, 236 *Haemophilus influenzae*, 176 *Streptococcus pyogenes*, 34 *Moraxella catarrhalis*, a total of 72 fungal isolates of the *Aspergillus* and *Candida* species and a small percentage of other bacteria. The *S. pneumoniae* strains isolated in the year 2000 exhibited high level resistance to penicillin at 5.7% and intermediate resistance at 31.4%; in the year 2001 the percentage of highly resistant isolates increased to 9.6% and the percentage of intermediately resistant strains rose to 39.5%. The *S. pneumoniae* erythromycin-resistant strains increased from 31.2% in 2000 to 41.3% in 2001; similarly, the sulphamethoxazole/trimethoprim-resistant strains increased from 24.3% in 2000 to 32.8% in 2001. Differences were also noted in *H. influenzae* resistance to sulfamethoxazole/trimethoprim, which increased from 7% in 2000 to 16.2% in 2001. The rest of the isolated pathogens did not show any significant difference in resistance, as expected.
Conclusions: No significant differences in the incidence of the main otic pathogens were found. A significant increase in sulfamethoxazole/trimethoprim resistance was noted in *H. influenzae* isolates. *S. pneumoniae* displayed significant increase in resistance to suggested antibiotics (penicillin, erythromycin, sulfamethoxazole/trimethoprim).

P1268 Antimicrobial susceptibility of enteric bacteria from chickens in Europe: results of the European Antimicrobial Susceptibility Surveillance in Animals (EASSA) program (1999–2001)

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Objectives: Antimicrobial resistance in enteric bacteria of food animals and the potential for transfer of resistance into the human population is a cause of concern. National surveillances of susceptibility in foodborne bacteria are common but international surveys are rare. EASSA is the first program to study the in vitro antimicrobial susceptibility of zoonotic and commensal bacteria of healthy animals (poultry, pigs, cattle) at slaughter across Europe. The survey included identical sampling and bacterial isolation procedures in eight countries, and MIC determination to panels of human antimicrobials. Here, the findings for chickens are reported.
Methods: Cecal samples were randomly collected by meat inspectors at four abattoirs per country in four countries. Each flock was sampled only once. Samples (usually 200 per country) were sent to national laboratories for isolation of *Campylobacter* spp., *Salmonella* spp. and *E. coli*. Isolates were sent to a central laboratory for agar dilution MIC testing (NCCLS, 1999). Resistance was based on NCCLS breakpoints, and was calculated for each drug and each country.
Results: The total number of strains isolated were 802, 120 and 546 for *E. coli*, *Salmonella* and *Campylobacter*, resp. The range in percentage resistance among the countries tested for *E. coli* to ampicillin (A), cefepime (CP), cefotaxime (CT), ciprofloxacin (C), chloramphenicol (CA), gentamicin (G), streptomycin (S), tetracycline (TE), and trimethoprim (TR) were 5–51, 0, 0, 0–4,

0.5–16.6, 0–4.5, 7–51, 6.5–85.4, and 2–49.7%, respectively. For *Salmonella* the resistance percentages were 0–54.7 (A), 0 (CP), 0 (CT), 0 (C), 0–14 (CA), 0–5.3 (G), 14–52 (S), 0–52 (TE), and 24–50 (TR), respectively. For *Campylobacter* the ranges were 0–35 (nalidixic acid), 0–34 (C), 0–3 (erythromycin), 0 (G), and 0–58 (TE)%. For *E. coli* Sweden had the lowest and France the highest levels of resistance (except for S). For *Salmonella* the low prevalence precluded a valid comparison among the countries; for *Campylobacter*, Sweden had 0% resistance; France and The Netherlands had similarly higher values.
Conclusions: This survey, based on identical methods across the EU, shows that the levels of resistance among enteric bacteria in chickens differ markedly geographically. This may reflect differences in antibiotic policies in individual countries. The results also suggest that there is a low level of resistance to antibiotics that are commonly used to treat foodborne disease in humans.

P1269 Epidemiology and erythromycin resistance of group A streptococci isolated from elementary school children and acute pharyngitis in Jinju, Korea

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Objectives: Group A streptococci (GAS), the most common cause of bacterial pharyngitis, has become more resistant to erythromycin (EM) or clindamycin (CC), as these antibiotics are widely used. The degree of resistance and its mechanism is not well known for these isolates in Korea.
Methods: Throat cultures were taken from 581 healthy elementary school children in 2002 and from 246 children of acute pharyngitis at a local clinic in the winter of 2001 and in the spring of 2002. T typing and emm genotyping was performed. Antibiotic sensitivity with disk diffusion, phenotypic distribution and resistance genes of EM resistance were investigated for the isolates.
Results: The isolation rate of GAS was 17% in the carriers and 50% in the acute pharyngitis. Although T12 is most common, 27.6% in the carriers and 37.4% in acute pharyngitis. Emm12 (33.7%), emm18 (9.2%), emm22 (8.2%) and emm1 (7.1%) were common in the carriers, while emm12 (28%), emm75 (18.4%), emm22 (12.8%) and emm2 (12%) were frequent in the pharyngitis. Resistance to EM and CC were 50 and 34%, respectively, in the carriers, compared with 46 and 20% in the acute pharyngitis. Constitutive resistance (CR) and M phenotype were 65 and 33% in the carriers, compared with 42 and 58% in the pharyngitis. The strains showing CR and M phenotypes were positive for *ermB* and *mefA* gene, respectively. Inducible resistance phenotype was rarely seen, being only 2% in the carriers. Eighty eight percent of *emm12* contained *ermB* gene in the carriers and 60% of *emm12* in the pharyngitis.
Conclusions: GAS is very common in school children and in acute pharyngitis. Emm12 is most common and it has high resistance to EM and CC. The antimicrobial resistance of GAS has become a significant problem in our community, which is higher in the carriers than in the pharyngitis. Precise diagnosis and adequate treatment for acute bacterial pharyngitis is needed.

P1270 Detection of CTX-M beta-lactamases in *Escherichia coli* strains from a hospital in Northern Italy

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Objectives: To investigate the presence of CTX-M-related enzymes in *Escherichia coli* clinical isolates producing extended-spectrum beta-lactamases (ESBLs), at the Pavia University Hospital.
Methods: Twenty-eight nonreplicate *E. coli* isolates with an ESBL-positive phenotype, collected from inpatients of the *S. Matteo* I.R.C.C.S. Hospital of Pavia (northern Italy) in the period January–November 2002, were investigated. MICs for cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP) and aztreonam (ATM) were determined by a microdilution test as recommended by NCCLS (Approved Standard M7-A4). ESBL production was detected by the application of BD-Phoenix ESBL screen flow, and by a double-disk synergy test between clavulanate or tazobactam and CTX, FEP, CAZ and ATM. Conjugal transfer of resistance determinants was tested in liquid medium. Beta-lactamases, separated by analytical isoelectric focussing (IEF), were detected using nitrocefin; their activity against CTX, CAZ, FEP and ATM was tested by a bioassay. PCR amplification of *blaCTX-M* alleles was carried out with primers designed on highly conserved gene regions. The nature of the gene was determined by amplicon sequencing. Pulsed-field gel electrophoresis (PFGE) profiles of genomic DNA digested with NotI were analyzed using the Bio-Rad Gene Path Procedure.
Results: Fifteen isolates were characterized by higher levels of resistance to cefotaxime than to ceftazidime. IEF of crude extracts of the 15 isolates revealed multiple beta-lactamase bands including a pI 8.4 band that, in a bioassay, was active on CTX, FEP and ATM. PCR screening for blaCTX-M determinants was positive in 12 isolates. Sequencing revealed the presence of blaCTX-M-1. The ESBL determinants were transferable by conjugation. Comparison of the PFGE profiles of CTX-M producers revealed clonal heterogeneity.
Conclusion: CTX-M-type ESBLs are emerging also in Italy, where they can contribute to beta-lactam resistance in nosocomial isolates of *E. coli*. Plasmid dissemination seems to be a major mechanism for the spread of these resistance determinants among nosocomial strains.

P1271 Epidemiology of bacteremic urinary tract infections and antimicrobial resistance from a French hospital network in 2001

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Objectives: To update antimicrobial guidelines based upon bacteremic urinary tract infections (BUTI) epidemiology and antimicrobial resistance.
Methods: A prospective survey was conducted in eight general nonteaching hospitals from Paris and area during 2001. BUTI was defined as bacteremia with a documented UTI origin, community and nosocomial acquired were differentiated based on historical data. Administrative data, characteristics of patients, clinical information, microorganism and their antimicrobial resistance (according to 'Comité de l'Antibiogramme de la Société Française de Microbiologie', http://www.sfm.asso.fr) were collected.
Results: Among 1777 bacteremia, 526 (30%) were BUTI. The BUTI yearly incidence was 0,37 per 100 admissions and 0.68 per 1000 days of hospitalization. Patients: (female: 62%) were admitted to the medicine wards (54%), surgery (17%), ICU (9%), emergency (7%), obstetric and pediatric (6%). BUTI were community-acquired (74% CA) or nosocomial (26% N). Patients ages (years) were:0–15:2%, 16–30: 7%, 31–45: 11%, 46–60 : 16%, 61–75: 26% and >75: 37%. Patients had a urinary catheter in 7% of CA BUTI and in 41% of N BUTI. Bacteria ($n = 528$) most frequently isolated were 402 *E. coli* (76%), 23 *P. mirabilis* (4%), 25 *Klebsiella* sp. (4%), 23 *S. aureus* (4%), 17 *E. fecalis* (3%). The resistance rates were as follows: Amk = Amikacin, Amx = Amoxicillin, Amc = Amx + clavulanic acid, BLSE = expanded spectrum beta lactamase Cip = Ciprofloxacin, Ctx = Cefotaxime, Gm = Gentamicin, Nal = Nalidixic acid, Tic = Ticarcillin. Among *S. aureus*, eight (35%) were methicillin R (4/9 N and 4/14 CA). No glycopeptide resistant enterococci or staphylococci were notified.

%	Amx	Tic	Amc	Ctx (BLSE+)	Gm	Amk	NaI	Cip
E. coli ($n = 402$)								
CA ($n = 362$)	49	46	38	1 (0)	0	0	9	5
N ($n = 71$)	51	50	45	4 (3)	4	0	17	12
Enterobacteria ($n = 471$)								
CA ($n = 362$)	55	51	43	1 (0)	3	1	10	5
N ($n = 109$)	61	55	42	6 (3)	3	2	22	15

Conclusion: Guidelines for N BUTI must consider the increased fluoroquinolones résistance rates. 3rd generation céphalosporins and aminosides remain the most active compounds for CA and N BUTI.

P1272 Epidemiologic evaluation of high-level gentamicin resistant (HLGR) among blood isolates of enterococci in the south-east of Sweden during 1994–2001

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Objectives: To investigate the epidemiology of HLGR among blood isolates of enterococci collected from patients admitted to three hospitals in the south-east of Sweden.

Methods: Three hundred and fifty-two (352) initial blood isolates of enterococci were collected from patients admitted to three hospitals in the southeast of Sweden between April 1994 and December 2001. No zone around a gentamicin 30-μg disc was defined as HLGR according to a disk diffusion method recommended by the Swedish Reference Group of Antibiotics (SRGA) (http://www.srga.se). Isolates with HLGR as well as 33 isolates To study similarity of the PFGE patterns between HLGR and no HLGR strains, 33 strains of *E. faecalis* with no HLGR, matched by year and unit with the HLGR *E. faecalis* isolates, were selected for detection of genetic relatedness. Detection of related clones was performed using pulsed-field gel electrophoresis of SmaI DNA macrorestriction fragments. The isolates with HLGR were included for PCR detection of the *aac(6′)Ie-aph(2″)Ia* gene. *E. faecalis* ATCC 51299 was included as positive control and *E. faecalis* ATCC 29212 as negative control.
Results: Of the isolates, 246 (70%) were *E. faecalis* and 106 (30%) *E. faecium*. Thirty-three (13%) of the *E. faecalis* isolates and four (3.8%) of the *E. faecium* isolates were HLGR. Of the *E. faecalis* with HLGR 22 (67%) belonged to the same clone. All the four *E. faecium* with HLGR had unique PFGE patterns. It was no similarity in any of the PFGE patterns between the *E. faecalis* with HLGR and the 33 tested strains of *E. faecalis* with no HLGR. All isolates with HLGR and the positive control (*E. faecalis* ATCC 51299) carried the *aac(6′)Ie-aph(2″)Ia* gene and the gene was not present in the negative control (*E. faecalis* ATCC 29212).
Conclusion: The results show genetic relatedness among 67% of the *E. faecalis* isolates with HLGR indicating a clonal spread in the three hospitals in southeast of Sweden.

P1273 Antimicrobial susceptibility and the molecular epidemiologic characteristics of *Neisseria gonorrhoeae isolates in Korea*

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Objectives: The prevalence of penicillinase-producing or fluoroquinolone (FQU)-resistant *Neisseria gonorrhoeae* varies greatly depending on countries. There are only few reports on the resistance mechanisms and molecular epidemiologic studies on Korean isolates. The aims of this study were (i) to determine the antimicrobial susceptibilities, (ii) to determine the mutations of the quinolone resistance-determining region (QRDR) of gyrA and parC of FQU-nonsusceptible isolates, and (iii) to conduct a molecular epidemiological analysis on the strains isolated from Korea and other countries.
Methods: The susceptibility of 453 Korean isolates of *N. gonorrhoeae* was tested by the NCCLS disk diffusion test and MICs for 49 isolates were tested by the agar dilution method. The PCR-products of QRDRs of gyrA and parC were used to detect presence of mutation by direct sequencing. Nhe I-digested genomic DNA of isolates from Korea and Western Pacific countries was separated by PFGE and the patterns were analyzed.
Results: Disk diffusion test showed none of the Korean isolates were susceptible to penicillin G and to tetracycline. Among the isolates, 63% were penicillinase-producing *N. gonorrhoeae* (PPNG). A marked rise in ciprofloxacin (CIP)-resistance rate was noted, from 16% in 1999 to 40% in 2001. The MIC50 of CIP, 0.5 mg/mL in this study, was an increase of 32- and 4-fold compared with those for isolates in 1993 and 1996, respectively. The MIC range of CIP was ≤0.008–32 mg/L for the non-PPNG strains, but was ≤0.008–0.5 mg/L for the PPNG strains, suggesting both resistances do not coexist. None were resistant to ceftriaxone and spectinomycin. However, the MIC of ceftriaxone ranged ≤0.008–0.12 mg/L. The MIC50 and MIC90 were, 0.03, and 0.06, respectively, which were 2-fold increase compared with the previous studies. The strains with a high-level CIP-resistance mostly carried a double amino acid substitution in gyrA and a single substitution in parC. CIP-resistant strains isolated from Korea and other Western Pacific countries showed different PFGE profiles, suggesting FQU-resistant strains emerged from the domestic clones.
Conclusions: In Korea, FQU-resistant *N. gonorrhoeae* isolates increased significantly, while PPNG remained prevalent. The existence of strains with decreased susceptibility to ceftriaxone and the high prevalence of strains resistant to FQU, indicate oral cephalosporins and FQU are no more suitable for empiric treatment of gonorrhea in Korea.

P1274 PER-1 ESBL production in a urinary isolate of *Providencia rettgeri*

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Objectives: There are a few number of extended-spectrum beta-lactamases (ESBLs) that are not closely related to TEM or SHV beta-lactamases. The PER-1 beta-lactamase, which is one of these, was first discovered in strains of *Pseudomonas aeruginosa* isolated from patients in Turkey. Later it was also found among isolates of Acinetobacter baumanni and some other Enterobacteriaceae taxa. Here we report PER-1 production in a urinary isolate of *Providencia rettgeri* strain.
Methods: A *P. rettgeri* strain was isolated from the urine of an elderly patient, with benign prosthatic hypertrophy, hospitalized at the Urology Clinic. Biochemical identification of the strain was made by API-20E (bioMerieux, Marcy I'Etoile, France). The susceptibility of the isolate was determined by the Kirby-Bauer disk diffusion assay and interpreted according to NCCLS. The presence of an ESBL was investigated by means of a double-disk synergy test agar, of the combined disk method and of *E*-test.
Results: Antimicrobial susceptibility testing of the strain revealed a high resistance to extended spectrum beta-lactams and intermediate resistance to aztreonam. The presence of an ESBL was suggested by observation of a synergy between the amoxicillin clavulanic acid disk and third generation cephalosporins and aztreonam. As PER-1 is widespread in Turkey, and besides *P. aeruginosa* and *A. baumanni* strains, is also found in Enterobacteriaceae like *Proteus mirabilis* and *Alcaligenes faecalis*, the strain was suspected to be harboring PER-1. PER PCR is performed, and blaPER is detected in the strain.
Conclusions: We describe for the first time the presence of PER-1 in *P. rettgeri*, an enzyme which is usually widespread among *A. baumanni* and *P. aeruginosa* in Turkey. To the best of our knowledge this report is the second description of an ESBL and first description of PER-1 production by *P. rettgeri*. Although *P. rettgeri* is usually susceptible to extended spectrum cephalosporins such as cefotaxime and ceftazidime, this strain warns us about the rapidly spreading ESBLs (including PER-1) amongst these strains.

P1275 Epidemiologic analysis of methicillin-resistant *Staphylococci* clones identified at a surgical hospital

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Objectives: To evaluate the prevalence and relatedness of clinical methicillin-resistant staphylococci (MRS) clones collected at a surgical hospital, using phenotypic and genotypic methods.
Methods: A total of 117 clones of MRS collected from clinical specimens of different inpatients within 1998–2002 were analyzed. All clones were characterized by conventional methods, according to the antimicrobial susceptibility patterns and genotypic profiles defined by hybridization with the mec A gene in PCR. For typing of *S. haemolyticus* and *S. aureus*, the macro restriction analysis of pulsed field gel electrophoresis (PFGE) and analysis of the PCR products of the coa gene were employed, respectively. Molecular typing was performed at the Robert Koch Institute, Wernigerode, Germany.
Results: From 117 MRS clones, 25 were coagulase-positive *S. aureus* and 92 coagulase-negative *Staphylococci*, including 64 cultures of *S. epidermidis* (sensu stricto), 17 – *S. haemolyticus*, 9 – *S. hominis*, 2 – *S. warneri*. All cultures were positive for the mec A gene. In the study of 10 strains of *S. hemolyticus* using SmaI macro restriction and PFGE, 2 clusters of completely identical strains were found. Each cluster consisted of two strains. The remaining strains were heterogeneous. The typing of the PCR products of the coa gene from 21 isolates of *S. aureus* revealed that 16 (80%) had similar patterns, while 2 and 1 isolates were different from this pattern. No correlation with the antibiograms of the clones in a cluster was registered.
Conclusion: Both phenotypic and genotypic methods are necessary for detection of the relatedness of clinical isolates.

P1276 Analysis of genetic background in *Staphylococcus aureus* isolates from North America producing toxic shock syndrome toxin 1

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Objectives: *S. aureus* producing toxic shock syndrome toxin 1 (TSST-1) are known to be the major cause of menstrually associated toxic shock syndrome. Nevertheless, important questions remain regarding the genomic diversity of these organisms, which appear to exhibit differences associated with global geographic origin and location. This issue was approached by an examination of TSST-1 producing *S. aureus* isolates from a variety of North American (U.S. and Canada) locations.
Methods: TSST-1 producing *S. aureus* isolates were obtained as part of a prevalence study culturing various body sites (i.e. nasal, anal, and vaginal) of women in five different North American locations (4 US. and 1 Canada). A total of 204 isolates were examined by macro-restriction (SmaI) analysis using pulsed field gel electrophoresis (PFGE). The isolates were additionally examined for methicillin resistance by agar disc diffusion using standard methods and by PCR and Southern hybridization analysis using mecA-specific primers and probes.
Results: Multiple isolates from the same individual generally exhibited highly related or identical PFGE patterns although there were exceptions. Common PFGE patterns were observed which were independent of geographic location or cultured body site. None of the isolates tested were identified as methicillin-resistant by disc diffusion and/or PCR-Southern hybridization analysis.
Conclusions: While significant genomic diversity occurs in North American TSST-1 producing *S. aureus* isolates, common geographically independent subtypes are present which are not associated with methicillin resistance. These data further reinforce previous reports regarding the tendency toward 'clonality' in TSST-1 producing *S. aureus* populations. In addition, the results aid in clarifying the infrequent association of methicillin resistance with menstrual TSS in North America which is in contrast to the situation reported in Europe and Japan.

P1277 Antimicrobial susceptibility patterns of Gram-negative bacteria in Swedish intensive care units

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Objective: To investigate the antibiotic susceptibility among Gram-negative isolates in Swedish intensive care units (ICUs).
Methods: During 2002 629 initial isolates of Gram-negative bacteria from 505 patients admitted to ICUs were collected at eight Swedish hospitals and minimal inhibitory concentration (MIC) determinations of ceftazidime (CAZ), ceftriaxone (CRO), cefuroxime (CXM), cefepime (CPM), ciprofloxacin (CIP), cotrimoxazole (TSU), gentamicin (GTM), imipenem (IPM), piperacillin (PIP), piperacillin/tazobactam (PTZ) were performed with *E*-test (AB Biodisk). An antibiotic to which >90% of isolates were susceptible according the breakpoints of the National Committee for Clinical Laboratory Standards (NCCLS) was defined as an treatment alternative (TA90) for empiric therapy.
Results: The sources of the isolates were respiratory tract (36%), urine (22%), gastrointestinal tract (17%), blood (10%) and skin and soft tissue (9%). The most frequently bacterial isolates were: *Escherichia coli* (27%), *Enterobacter* spp. (13%), *Klebsiella* spp. (12%), *Pseudomonas aeruginosa* (11%), *Haemophilus* spp. (6%), *Stenotrophomonas maltophilia* (5%), *Proteus* spp. (4%), *Acinetobacter* spp. (3%), *Citrobacter freundii* (2%) and *Morganella morganii* (2%). The number of TA90s were low (2) for *S. maltophilia* (CPM, TSU), higher (5–7) for *Enterobacter* spp./*C. freundii* (CPM, CIP, GTM, IPM, TSU), *P. aeruginosa* (CAZ, CPM, GTM, PIP, PTZ), *K. oxytoca* (CTZ, CPM, CIP, GTM, IPM, TSU), *Acinetobacter* spp. (CTZ, CPM, CIP, GTM, IPM, PTZ, TSU) and highest (8–10) for *K. pneumoniae* (CTZ, CPM, CRO, CIP, GTM, IPM, PTZ, TSU), *E. coli* (CTZ, CPM, CRO, CXM, CIP, GTM, IPM, PTZ), *M. morganii* (CTZ, CPM, CRO, CIP, GTM, IPM, PT, PTZ, TSU) and *Proteus* spp. (CTZ, CPM, CRO, CXM, CIP, GTM, IPM, PT, PTZ, TSU). When all isolates were pooled the TA90s were six (CTZ, CPM, CIP, GTM, IPM, PTZ).
Conclusions: Rather high frequencies of decreased susceptibility were seen to CRO, CXM, PIP and TSU. Higher number of isolates of *E. coli* and *Klebsiella* spp. with MIC > 1 mg/L to one or more 3rd generation cefalosporins suggest an increase in prevalence of extended spectrum beta-lactamases (ESBLs) than seen in earlier Swedish studies. This study also shows higher number of isolates of *P. aeruginosa* with decreased susceptibility to CIP (10%) and IMP (19%) than seen earlier in Sweden.

P1278 Macrolide-resistant pneumococci in Finland

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Objectives: Macrolide resistance is an increasing problem among pneumococci. We have studied prevalence of macrolide-resistant pneumococci (MRP), the connection between outpatient macrolide consumption and resistance, and macrolide resistance mechanisms.
Methods: FiRe (Finnish Study Group for Antimicrobial Resistance) data was used to determine the overall macrolide resistance rate and for determining the connection between macrolide use and resistance in Central Hospital Districts. The figures of the drug use were obtained from the National Agency of Medicines. Among the material of 2500 invasive/penicillin nonsusceptible (PNS) pneumococci, macrolide resistance was studied and resistance mechanisms were determined. Serotyping was performed in Oulu.
Results: Macrolide use in 1995–96 correlated highly significantly ($P = 0.007$) with macrolide resistance in 1997 among Finnish Central Hospital Districts. In 1997, macrolide resistance was 7% and in 2000, 11%. Among invasive pneumococci, 6% were MRP in 1998–2001, and macrolide resistance was most common resistance-type. Among the PNSP, macrolide resistance increased from 29 to 61% during 1996–2000. In the invasive material, *mef(A)* (M-phenotype) was the most common resistance gene and among PNSP, *erm(B)* (MLSB phenotype) was the most common. In a few percent of the MRP, a novel MS phenotype was seen. Among those, mutations in 23S rRNA or ribosomal proteins L4 and L22 were detected. Serotype 23 was the most common among the MRP.
Conclusions: Due to the high-quality resistance surveillance in Finland, we were able to study the relation between the regional drug use and resistance. The positive correlation gives more evidence for the common hypothesis that the spread of resistance can partly be controlled by the drug use. Macrolide resistance is not common among the invasive pneumococci, but still the commonest. For infections caused by the PNSP, macrolides should not be used, as among those, macrolide resistance reached 61% in 2000. Also, macrolide resistance among those is of high-grade, as it is often mediated by methylase gene.

P1279 Antimicrobial resistance of *Staphylococcus* in Spain: five nationwide prevalence studies

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Objectives: Data regarding the evolution of *Staphylococcus* resistance in a whole country have a definite influence in the design of empirical treatment regimens. However, incidence studies over long periods of time are expensive an very difficult to carry out. In order to ascertain the present situation of the antimicrobial resistance of *Staphylococcus* in our country and the evolution of the resistance, we performed five point prevalence studies over a 17-year period.
Methods: From 1986 to 2002 we carried out five point prevalence studies in a large group of Spanish hospitals (from 68 institutions in 1986 to 143 in 2002) collecting all *Staphylococcus* isolates in a single day. All microorganisms were identified in the five studies at the same laboratory, and antimicrobial susceptibility testing was performed against 15 antimicrobial agents using and automated microdilution method (MicroScan).
Results: The evolution in time of percentages of resistance against selected antimicrobials is summarized in Table 1 (PEN = penicilin, OXA = oxacillin,

Table 1 Percentage of resistance of *S. aureus*/coagulase negative staphylococci (CNS) to selected antimicrobials

Year	PEN	OXA	ERY	GEN	RIF	T/S	CIP	VAN	TEI
1986	95/86	1.5/32	7/41	5/25	1/4	–	0.6/1	0/0	0/0
1991	97/91	11/25	24/45	14/40	5/8	–	16/21	0/0	0/0
1994	92/77	17/34	34/58	19/49	8/9	0.5/22	20/24	0/0	0/0
1996	92/86	18/51	24/54	16/40	7/9	1/31	20/33	0/0	0/1
2002	88/79	30/63	33/64	18/35	2/7	2/22	37/50	0/0	0/0

ERY = erythromycin, GEN = gentamicin, RIF = rifampin, T/S = trimethoprim/sulfamethoxazole, CIP = ciprofloxacin, VAN = vancomycin, TEI = teicoplanin).
Conclusions: Over the period of study there was an overall resistance increase to most antimicrobials, mainly of *S. aureus* to oxacillin; resistance of *S. aureus* to T/S was anecdotal. In general all the isolates were uniformly susceptible to glycopeptides. Periodical performance of prevalence studies is an useful, unexpensive and easy tool to know the nationwide situation of a microorganism and its resistance to antimicrobials, provides important information about the changing spectrum and regional variation of antimicrobial resistance, and assists in the design of appropriate measures for controlling the emergence and spread of antimicrobial resistance.

P1280 Serotype distribution and evolution of antibiotic resistance among *Salmonella* isolates in a Greek hospital

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Objective: To determine the serotype distribution and to analyze the evolution of the antimicrobial resistance of *Salmonella* strains isolated from diarrheal patients in Crete, Greece, during the last three years (2000–2002).
Methods: A total of 251 *Salmonella* strains isolated from stool specimens were studied. Identification was made according to standard microbiological procedures and serotyping was performed with commercial antisera by the slide agglutination method. Antimicrobial susceptibility testing to seven antibiotics was checked by the disk diffusion method following the recommendations of the NCCLS.
Results: Two hundred and fifty-one *Salmonella* strains were isolated during the 3-year study period, which corresponded to 16 serotypes. *Salmonella enterica* serotype enteritidis was the most frequent (164 strains) accounting for 65.3% of the *Salmonella* isolated. *Salmonella enterica* serotype typhimurium (38 strains) constituted 15.1%, while the remaining 14 serotypes were rare, constituting 19.6% of the strains. Eleven percent of the isolates were resistant to ampicillin. There was a marked decrease in the resistance to this antibiotic during the study period. Between 2000 and 2001 ampicillin resistance decreased from 19.6 to 12.8%, while during 2002 only 5% of the isolates were resistant to this antibiotic. Eight percent were resistant to chloramphenicol (17.8% during 2000, 8.5% during 2001 and 2% during 2002), 20.3% to tetracycline (44.6% during 2000, 23.4% during 2001 and 4% during 2002). Four percent of the isolates were resistant to cotrimoxazole (7.1% during 2000, 4.2% during 2001 and 1% during 2002), 1.6% to gentamicin (5.3% during 2000, 0% during 2001 and 1% during 2002). Only three isolates were resistant to norfloxacin and one to ciprofloxacin.
Conclusions:
1. *Salmonella enterica* serotype enteritidis continuous to be the predominant *Salmonella* serotype in our region.
2. Resistance of *Salmonella enterica* to antimicrobial agents decreased the last 3 years, probably due to the more prudent use of the antibiotics.

P1281 In vitro activities of 17 antimicrobials against 200 clinical strains of *Streptococcus pneumoniae* isolated in Crete, Greece

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Objective: To study the in vitro activity of 17 antibiotics against 200 strains of *Streptococcus pneumoniae* isolated from clinical specimens in the University Hospital of Crete, Greece.
Material and methods: Susceptibility tests were done by the *E*-test method according to NCCLS guidelines. The following antibiotics were tested: penicillin G, cefuroxime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, erythromycin, clarithromycin, ciprofloxacin, levofloxacin, sparfloxacin, moxifloxacin, chloramphenicol, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin.
Results: The MIC90 (mg/L) and percentage of resistance (%) were as follow: penicillin G 0.023 (38%; 16.5% resistant and 21.5% intermediate), cefuroxime three (25.5% resistant and 5% intermediate), cefotaxime 0.75 (3.5% resistant and 14% intermediate), ceftriaxone 0.75 (2.5% resistant and 9% intermediate), cefepime 1.5 (12% resistant and 13.5% intermediate), imipenem 0.25 (1.5% resistant and 20.5% intermediate), meropenem 0.25 (1% resistant and 9% intermediate), erythromycin 32 (36%), clarithromycin 32 (36%) chloramphenicol 3 (4.5% resistant and 1% intermediate), tetracycline 16 (27.5% resistant and 2.5% intermediate), trimethoprim/sulfamethoxazole 12 (18.5% resistant and 16% intermediate), ciprofloxacin 2 (1%), levofloxacin 1.5 (0%), sparfloxacin 0.5 (1% resistant and 3.5% intermediate), moxifloxacin 0.125 (0.5%), and vancomycin 0.5 (0%). Multiple resistance was observed for 61 strains.
Conclusion: The high incidence of antibiotic-resistant pneumococci in our region underline the need for continuous surveillance of antimicrobial resistance profiles of *S. pneumoniae.*

P1282 The use of amoxicillin as selection pressure for trimethoprim resistance

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Objectives: To determine the impact of other antibiotic usage on the persistence of trimethoprim resistance in urinary tract isolates.
Methods: Antibiotic resistance profiles for urinary isolates of Lactose Fermenting Coliforms were reviewed to establish linked resistance patterns. GP prescribing data were analyzed and the impact of different classes of antibiotics on trimethoprim resistance was assessed using logistic regression (minitab). Gp prescribing habits were assessed using a questionnaire survey.
Results:
- 57% of questionnaires were returned. 80% of respondents use trimethoprim as first line treatment for urinary tract infection.
- 75% of respondents use amoxicillin as first line treatment for respiratory tract infections.
- 57% of all lactose fermenting coliform isolates from urine samples were resistant to trimethoprim.
- 25% of these isolates were resistant to both amoxicillin and trimethoprim (sometimes with other classes as well).

The commonest resistance pattern being amoxicillin and trimethoprim at 15%. Logistic regression showed an association between amoxicillin usage and percentage of isolates resistant to trimethoprim.
Conclusions: There is pressure to reduce the amount of antibiotic prescribing in general practice and to move away from agents with high levels of resistance. With an expectation that resistance will reduce over time. When developing a prescribing policy for general practitioners it is essential that associated resistance determinants are identified so that other factors associated with persisting resistance can be considered and modified at the same time. In this example reducing trimethoprim prescribing alone will not significantly impact on the level of trimethoprim resistance. Levels of amoxicillin prescribing will also have to be addressed.

P1283 Lack of evolution of resistance to teicoplanin or vancomycin of *S. epidermidis*: strains isolated in Nantes from 1990 to 2001

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Objectives: Following concerns about the potential development of resistance to teicoplanin and vancomycin in *Staphylococcus epidermidis*, this study was performed to track the possible evolution of resistance of strains isolated over a 11-year period in Nantes. A comparison was also made of results obtained with two different Mueller-Hinton (MH) liquid sensitivity test media.
Methods: 50 strains, randomly selected from all *S. epidermidis* strains isolated in Nantes over each 2-year period between 1990 and 2000, and 246 strains isolated in 2001 were tested for Minimum Inhibitory Concentrations (MIC) using both Difco and Bio-Rad MH broths. NCCLS methodology was used with the following breakpoints applied for teicoplanin: $S \leq 8$ mg/L, $I = 16$ mg/L, and $R \geq 32$ mg/L and vancomycin: $S \leq 4$ mg/L, $I = 8$–16 mg/L, and $R \geq 32$ mg/L.
Results: All isolates tested were deemed fully sensitive to vancomycin using both media. Teicoplanin summary results are shown in Table 1.

Table 1

Teicoplanin sensitivity	% of strains, D = Difco, B = Bio-Rad													
	1990		1992		1994		1996		1998		2000		2001	
	D	B	D	B	D	B	D	B	D	B	D	B	D	B
S	96	90	96	74	92	86	92	70	100	88	98	68	96	85
I	2	8	2	20	4	4	6	22	0	12	2	26	4	14
R	2	2	2	6	4	10	2	8	0	0	0	6	0	1

The number of *S. epidermidis* strains resistant to teicoplanin can be seen to have not increased significantly over the last 11 years, and there were actually fewer isolated in 2001 than in 1990. Bio-Rad liquid MH medium gave consistently higher MICs to both vancomycin and teicoplanin, although the latter was more greatly affected. With Difco media, the number of strains that were fully sensitive to teicoplanin ranged from 92 to 100%, whereas with Bio-Rad the range was 68–90%.

Conclusions: There is no evidence for an increase in resistance to teicoplanin or vancomycin of *S. epidermidis* since 1990. MICs of teicoplanin are more greatly affected by the media used than those of vancomycin. Bio-Rad MH broth gave consistently higher MICs than those with Difco MH broth. Depending on the media used, it is likely that teicoplanin MICs will vary; without this necessarily being the result of development of resistance.

P1284 *ypdI* gene involved in exopolysaccharide production in *Escherichia coli* K-12 CM2555 mutant

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Objectives: We are well aware of the increasing microbial resistance to antibacterial agents such as antibiotics and biocides. A significant issue in the phenomenon of resistance is the presence of biofilms. By forming such sessile, multiorganism communities, the pathogens are better equipped to counteract the action of commonly used antibacterial agents than when living in a planktonic state. Exopolysaccharides comprise an important component of biofilms. Although not always essential in the early stages of biofilm formation, they are imminent in sustaining the biofilm architecture, being a matrix in which the bacteria are embedded and which, in part, protects them from the action of caustic agents. *Escherichia coli* K-12 is a commonly researched model bacterium, a useful tool in elucidating many metabolic processes. Although *E. coli* exopolysaccharide production has been extensively examined, many issues still remain unclear. Under standard laboratory conditions, *E. coli* strains produce only basic levels of the main exopolysaccharide colanic acid. In our laboratory, we have observed that an *E. coli* mutant, strain CM2555, formed mucoid colonies when bearing a multicopy plasmid pJPA2.5 carrying a 3.6-kb fragment of *E. coli* chromosome. The aim of this work was to map gene(s) responsible for the unusual mucoid colony formation and to investigate the nature of the slime.

Methods: To reach our objectives we used a genetic mapping approach, including DNA cloning techniques and polymerase chain reaction. In order to investigate the nature of the slime, we analyzed bacterial protein profiles by polyacrylamide gel electrophoresis. Gas chromatography in conjunction with mass spectrometry was performed.

Results: As a result of systematic mapping of the gene(s) responsible for mucoid colony formation of *E. coli* CM2555 strain we discovered that the phenotype was due to an increased copy number of *ypdI* gene. This gene's functions have not been heretofore experimentally examined. The slime accompanying mucoid colony formation is not associated with significant changes of bacterial protein profile. The mucoidy results from elevated production of extracellular polysaccharide colanic acid.

Conclusions: This work shows for the first time that the *ypdI* gene is involved in colanic acid synthesis in *E. coli*. However, this effect seems to be genetic background specific since none other common *E. coli* wild-types tested displayed changed colony morphology after introducing *ypdI* on a multicopy plasmid.

P1285 Molecular fingerprinting of multidrug-resistant isolates of *Mycobacterium tuberculosis from Chest Diseases Hospital in Kuwait*

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Objectives: To perform molecular fingerprinting on 21 consecutive multidrug-resistant *Mycobacterium tuberculosis* strains isolated from tuberculosis (TB) patients at the Chest Diseases Hospital, Kuwait, during 1998–2001.

Methods: The genotyping was performed by touchdown double-repetitive-element-PCR (DRE-PCR). The genotypic relationships among isolates exhibiting cluster pattern was further studied by determining the presence of distinct mutations in rifampin-resistance-determining region (RRDR) of the *rpoB* gene, the presence of R463 or L463 polymorphism at codon 463 in the *katG* gene and the presence or absence of S315T mutation (the most common genetic alteration causing resistance to isoniazid) in the *katG* gene.

Results: The isolates exhibited 13 distinct patterns in DRE-PCR with more than half of the isolates (12 of 21) exhibiting unique patterns. All the isolates (12 of 12) yielding two or more DNA fragments in DRE-PCR were unique strains. The remaining 9 isolates yielded a single DNA fragment of the same size in DRE-PCR. The data on the presence of distinct mutations in RRDR of the rpoB gene, the presence of R463 or L463 polymorphism at codon 463 in the katG gene and the presence or absence of S315T mutation in the katG gene showed that 6 of 9 cluster pattern isolates were genotypically distinct strains.

Conclusions: The data suggest that most expatriate patients were infected with a unique strain of *M. tuberculosis* and that the apparent grouping of some cluster pattern isolates was most likely due to low copy number of IS6110 rather than any specific relationship among these isolates. The results may also have implications for tuberculosis control measures in low incidence countries with large immigrant populations.

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P1286 Antimicrobial resistance among *Campylobacter jejuni/coli* strains acquired in Sweden and abroad

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Objectives: The objective of this study was to find out whether there has been a difference in antibiotic resistance among *C. jejuni/coli* strains acquired outside Sweden and domestically during the period 1990–2002.

Methods:

1. Quantitative data from susceptibility tests of *C. jejuni/coli* were obtained from the database in the department of Clinical Microbiology, Växjö, Sweden.
2. Breakpoints, distinguishing wild type populations from those not fully susceptible, were determined from zone histogram analysis.
3. Information about the countries, where the *C. jejuni/coli* strains most probably were contracted, was obtained from the notifications sent to the department of Communicable Disease Control and Prevention, Växjö, Sweden.

Results: Data on antibiotic susceptibility and country where the strains were contracted was obtained from 1419 of 1545 *C. jejuni/coli* cases (92%). In 575 of the 1419 cases the strains were contracted in Sweden and in 844 cases in countries outside Sweden. During the study period, a steady trend towards increasing resistance to tetracycline (from 20 to 50%) and ciprofloxacin (from 0 to 60%) was seen in *C. jejuni/coli* contracted outside Sweden. No significant resistance to tetracycline or ciprofloxacin was seen in the domestically acquired strains. No significant resistance to erythromycin was seen either in domestically acquired *C. jejuni/coli* strains or strains acquired abroad, during the study period.

Conclusions: A clear trend over a 13-year period of increasing ciprofloxacin and tetracycline resistance, among *C. jejuni/coli* strains acquired abroad, was evident. This trend was not seen for erythromycin or in domestically acquired strains.

P1287 *Enterococcus* spp. organisms isolated in a teaching hospital. A 2-year bacteriologic survey

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Objective: To assess the epidemiology and resistance features of enterococci (E) cultured in a 2-year period, with attention on the difference between *E. faecalis* and *E. faecium*, and the role of novel antibiotics targeted on multi-resistant Gram-positive cocci: dalfopristin-quinupristin (DQ), linezolid and fluoroquinolones.
Methods: Results: 4628 *E. faecalis* strains and 648 *E. faecium* isolates were examined. An increase of *E. faecium* vs. *E. faecalis* was observed in the year 2001 ($P < 0.0001$), with growing involvement of cardiovascular ($P < 0.0001$), intrabdominal ($P < 0.0001$), and genital tract ($P < 0.003$). *E. faecalis* showed a higher in vitro sensitivity than *E. faecium* against penicillin, ampicillin, nitrofurantoin, levofloxacin and streptomycin ($P < 0.0001$). On the other hand, *E. faecium* was more susceptible to gentamycin and DQ ($P < 0.0001$). The activity of tetracyclines (30% of strains), linezolid (99%) and glycopeptides did not show significant differences between *E. faecalis* and *E. faecium*. Glycopeptides and linezolid proved the most effective drugs on E, since only 2 *E. faecalis* strains tested resistant, while all the 648 *E. faecium* strains were sensitive. When comparing the susceptibility pattern of the year 2001 vs. 2000, no significant difference was found for both E, which showed a slightly increased sensitivity to penicillin, semisynthetic penicillins, gentamycin, streptomycin, nitrofurantoin and linezolid for *E. faecalis*, and penicillin, tetracyclines and linezolid for *E. faecium*.
Conclusion: Susceptibility levels of E show broad variations according to literature studies, due to the large spectrum of involved variables. In our experience based on >5000 E strains, a temporal increase of isolations, and an increasing role of *E. faecium* were noticed. The in vitro studies confirmed a different profile of *E. faecalis* vs. *E. faecium*. Penicillin G and semisynthetic penicillins are still highly active on *E. faecalis*, but it is not true for *E. faecium*. The continued in vitro efficacy of nitrofurantoin, gentamycin and streptomycin is of interest. Among recently introduced compounds, linezolid is effective on 99% of strains and maintains a very high activity on the emerging *E. faecium*.DQ and levofloxacin are characterized by a greater activity on *E. faecium* and *E. faecalis*, respectively. Glycopeptides showed resistance levels lower vs. those observed in most literature surveys, and remain the reference molecules for all E. The emerging of resistances against recent molecules suggests a prudent use of all available therapeutic resources.

Staphylococci

P1288 Antimicrobial in vitro activity of quinupristin/dalfopristin against staphylococci and comparison to other antimicrobial agents

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Objectives: To survey the current in vitro resistance of quinupristin/dalfopristin (Synercid: SYN) against coagulase negative staphylococci (CoNS) and *S. aureus* and also to compare the resistance of SYN to the resistance of oxacillin (OX), erythromycin (ER), clindamycin (CL) and norfloxacin (NOR).
Methods: During 2002, 690 clinical isolates of CoNS and 66 clinical isolates of *S. aureus* were collected in our hospital. Cons strains were isolated: 162 from urine cultures, 183 from blood cultures and 345 from wound infections. *S. aureus* strains were isolated: 6 from urine cultures, 15 from blood cultures and 45 from wound infections. Identification was carried out by ID 32 Staph for CoNS and by conventional methods for *S. aureus*. Resistance was determined by the breakpoint system: mini API (bioMerieux).
Results: All CoNS and *S. aureus* strains were susceptible to vancomycin. Ten percent (69/690) of CoNS and 9% (6/66) of *S. aureus* strains were resistant to quinupristin/dalfopristin. The resistance of CoNS strains to other antimicrobial agents was: 64% (444/690) to oxacillin, 60% (414/690) to clindamycin, 73% (507/690) to erythromycin and 43% (300/690) to norfloxacin. The resistance of *S. aureus* strains to other antimicrobial agents was: 27% (18/66) to oxacillin and norfloxacin, 32% (21/66) to clindamycin, 59% (39/66) to erythromycin.
Conclusions: In vitro activity of SYN against CoNS (susceptibility: 90%) and *S. aureus* (susceptibility: 91%) is indicative that SYN is an effective antibiotic to staphylococcal infections and its superiority against OX, ER, CL and NOR makes it an alternative therapy in staphylococcal infections.

P1289 Antibiotic-induced stress and the expression of cell wall components by strains of *Staphylococcus aureus* displaying increased resistance to vancomycin (VISA)

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Background: Some antibiotics are known to alter bacterial structure at subgrowth inhibitory concentrations (sub-MIC) and in so doing alter their susceptibility to phagocytosis. Vancomycin intermediate resistant *S. aureus* (VISA) display thickened cell walls and reduced levels of protein A.
Objective: To examine the effect of sub-MIC linezolid (LZD), synercid (SYN) and daptomycin (DAP) on surface topography of VISA.
Methods: Six strains of VISA were grown for up to 16 h in the presence or absence of sub-MIC antibiotic, examined using transmission electron microscopy for cell wall thickness and measured by ELISA for protein A content. Susceptibility of antibiotic-exposed bacteria to phagocytosis by isolated human neutrophils was examined in relation to surface changes in the bacteria.
Results: VISA exposed to sub-MIC LZD and SYN had thickened cell walls (increased by >50%) as compared with no-drug controls whereas DAP-treated cells were thinner (decreased by >30%). Most VISA elaborated little or no protein A but in those that did, levels were reduced significantly (<10 ng/100 million cells) in LZD- and SYN-exposed bacteria. For some strains, LZD- and SYN-staphylococci were more susceptible to opsonophagocytosis. There was no difference between antibiotic-exposed VISA and VISA grown in the absence of any antibiotic in terms of their ability to induce neutrophil respiratory burst.
Conclusion: Whereas exposure to several antibiotics at sub-MIC levels altered the surface appearance of VISA, the changes were not translated uniformly into altered susceptibility to phagocytosis; there was no common pattern among the six strains of VISA tested.

P1290 A study of the effects of media and methodology for sensitivity testing of 116 strains of apparently teicoplanin-resistant staphylococci from South Africa

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Objectives: One hundred and sixteen strains of *Staphylococcus* spp. from South Africa were deemed teicoplanin-resistant using brain heart agar media containing 12 mg/L of teicoplanin. This study determined the sensitivity of these strains when tested using different methodologies and batches of Mueller-Hinton (MH) media.
Methods: Identities of strains were confirmed and methicillin-resistance determined by disk agar diffusion. Teicoplanin (30 µg) and vancomycin (5 µg) disk sensitivities were performed along with other relevant antibiotic disk sensitivities. Agar and broth dilution MICs to teicoplanin and vancomycin were determined using MH agar from two sources: Difco and Bio-Rad, and MH broth from Difco. Sensitivity test methods were as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) with ATCC strains as controls. Doubling dilutions ranged from 0.016 mg/L to 64 mg/L, and inoculum was 5×10^3–10^4 cfu. Results were read after incubation at 35°C for 20–24 h. NCCLS breakpoints for teicoplanin, for MIC and disk diameter, respectively, are: sensitive, <8 mg/L and >14 mm, intermediate, 16 mg/L and 11–13 mm, and resistant, >32 mg/L and <10 mm.

Results: One hundred and seven out of 116 strains were methicillin-resistant and 101/107 also had kanamycin, tobramycin, gentamicin and rifampicin resistance phenotypes. Inhibition zones for disk testing against teicoplanin and vancomycin were >16 mm and >18 mm, respectively, for all except five strains of *S. haemolyticus* (all >14 mm for teicoplanin). MIC50/90 s are shown in the table.

	Teicoplanin			Vancomycin MIC 50/90 (mg/L)		
Species	Difco broth	Difco agar	Bio Rad agar	Difco broth	Difco agar	Bio Rad agar
S. aureus ($n=40$)	2/4	4/4	8/8	½	2/2	4/4
S. epidermidis ($n=34$)	4/8	4/8	8/16	2/2	2/2	2/2
S. haemolyticus ($n=39$)	4/16	4/16	16/32	½	2/2	2/4
Staph. spp ($n=3$)	All MICs<4	All MICs <4	All MICs < 16	All MICs 2	All MICs2	All MICs 2

Intermediate or resistant MICs to teicoplanin were obtained with 36 strains (only one *S. aureus*) using Bio-Rad MH agar, compared with only eight (two *S. epidermidis*, six *S. hemolyticus*) using Difco broth.
Conclusions: All these strains were considered to be resistant to teicoplanin when tested in South Africa. However, when retested none of the staphylococci were resistant to teicoplanin using Difco broth, as recommended by the NCCLS, whereas seven strains (one *Sepidermidis*, six *Shaemolyticus*) were determined as resistant with Bio-Rad MH agar. As the table shows, the variation in results with different media demonstrates that careful adherence to standardized methods is required.

P1291 Sensitivity testing of teicoplanin against *S. epidermidis*: the impact of different media on MICs

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Objectives: Following the introduction of the VITEK 2 for susceptibility testing in our laboratory, some strains of *S. epidermidis* were determined as having higher than expected MICs to teicoplanin, even though by disk diffusion they appeared fully sensitive. This study aimed to see if 'resistance' was affected by the method used to define MIC.
Methods: Two hundred and forty-six *S. epidermidis* strains isolated in Nantes in 2001 and for which MICs to teicoplanin and vancomycin had been estimated using VITEK 2 were retested in Mueller-Hinton (MH) agar media from Difco, Oxoid, Becton Dickinson, bioMérieux and Bio-Rad and also in liquid MH media from Difco, Oxoid, Becton Dickinson and Bio-Rad. NCCLS methodology was used with the following breakpoints applied for teicoplanin: S ≤ 8 mg/L, I = 16 mg/L, and R ≥ 32 mg/L and vancomycin: S ≤ 4 mg/L, I = 8–16 mg/L, and R ≥ 32 mg/L.
Results: The strains were analyzed separately according to methicillin sensitivity: 52 strains were methicillin-sensitive (MSSE) and 194 resistant (MRSE). All MSSE and 99–100% (depending on media) of MRSE were fully sensitive to vancomycin. The table gives teicoplanin sensitivity test results.

	Sensitivity test medium (A=agar, B=broth) (% of strains, MSSE/MRSE)									
Teico test result	Difco A	Oxoid A	BD A	bioM A	BioRad A	Difco B	Oxoid B	BD B	Bio Rad B	Vitek
S	100/99	100/97	98/89	100/96	90/66	100/99	100/95	100/99	94/82	98/90
I	0/1	0/2	0/9	0/4	10/31	0/1	0/40	0/1	6/16	2/9
R	0/0	0/1	2/2	0/0	0/3	0/0	0/1	0/0	0/2	0/1

Tests performed with MH agar from Difco, Oxoid, bioMérieux and MH broth from Difco, BD and Oxoid, all resulted in 90–100% of strains being considered sensitive. Vitek, BD agar and Bio-Rad agar and broth all gave higher MICs. In general, 98–100% MSSE were sensitive and 89–99% of MRSE were sensitive, except when tested with Bio-Rad agar and broth (66 and 82%, respectively). VITEK 2 gave MICs in the higher range but which were closer in values to most of the other media used.
Conclusions: The variety of test media gave a range of results where the percentage of strains of *S. epidermidis* considered resistant to teicoplanin ranged from 0 to 3%, while those evaluated as intermediate ranged from 0 to 10% for MSSE and 1–31% for MRSE, which clearly indicates that great caution should be used with some test media. VITEK 2 gave MICs on the slightly high side and Bio-Rad MH agar and broth would appear to be giving results that do not concur with other MH media.

P1292 In vitro activity of linezolid in comparison with vancomycin, quinopristin/dalfopristin, levofloxacin, and moxifloxacin to clinical isolates of staphylococci

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Objectives: Antimicrobial resistance among Gram-positive bacteria has increased and new antibiotics have been introduced during the last years. The aim of the study was therefore to evaluate the in vitro activities of linezolid (LIZ) in comparison with vancomycin (VAN), quinopristin/dalfopristin (SYN), levofloxacin (LFX), and moxifloxacin (MOX) to staphylococci.
Methods: A total of 7245 clinical isolates of patients of a university hospital were investigated in a prospective study over a time period of 24 months. The isolates had been identified as pathogens, not as part of the normal flora. They included 2072 methicillin-susceptible *S. aureus* (MSSA), 212 methicillin-resistant *S. aureus* (MRSA), 868 methicillin-susceptible *S. epidermidis* (MSSE), 1015 methicillin-resistant *S. epidermidis* (MRSE), 198 methicillin-susceptible *S. haemolyticus* (MSSH), 439 methicillin-resistant *S. haemolyticus* (MRSH), 304 methicillin-susceptible strains of other coagulase-negative staphylococci (MSCoNS), and 137 methicillin-resistant strains of other coagulase-negative staphylococci (MRCoNS). The MIC-values were determined by broth microdilution assay (MICRONAUT-S-GENARS, Merlin, Bornheim-Hersel, Germany.
Results: MIC50 and MIC90 (mg/L) were:

- Liz: 1/2 (MSSA), 1/2 (MRSA), 0.5/1 (MSSE), 0.5/1 (MRSE), 0.5/1 (MSSH), 0.5/1 (MRSH), 0.5/1 (MSCoNS), 0.5/1 (MRCoNS)
- Van: 1/1 (MSSA), 1/1 (MRSA), 1/1 (MSSE), 1/2 (MRSE), 0.5/1 (MSSH), 1/2 (MRSH), 0.5/1 (MSCoNS), 1/2 (MRCoNS)
- Syn: 0.5/1 (MSSA), 1/1 (MRSA), 0.5/0.5 (MSSE), 0.5/0.5 (MRSE), 0.5/1 (MSSH), 0.5/1 (MRSH), 0.5/1 (MSCoNS), 0.5/1 (MRCoNS)
- Lfx: 0.125/2 (MSSA), 4/16 (MRSA), 0.25/8 (MSSE), 4/8 (MRSE), 0.125/8 (MSSH), 4/16 (MRSH), 0.25/1 (MSCoNS), 4/16 (MRCoNS)
- Mox: 0.063/1 (MSSA), 2/4 (MRSA), 0.125/2 (MSSE), 1/2 (MRSE), 0.125/2 (MSSH), 1/4 (MRSH), 0.125/0.5 (MSCoNS), 1/4 (MRCoNS).

Conclusions: Apart from some elevated MIC-values, espicially of LFX and MOX, the antimicrobials tested exhibited good activities to methicillin-susceptible as well as methicillin-resistant staphylococci. No resistance to LIZ could be detected.

P1293 In vitro activity of six novel antimicrobial agents against staphylococci using the Phoenix™ Automated Microbiology System

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Objectives: Staphylococcal resistance to existing antimicrobial agents has led to the need for increased surveillance methods, strict control measures and the development of new antimicrobics to combat this escalating trend. In this study, six new antimicrobics, Linezolid (LZD), Daptomycin (DAP), Garenoxacin (GRN), Gemifloxacin (GEM), Ertapenem (ETP) and Telithromycin (TEL), were evaluated for in vitro activity in the Phoenix ™ Automated Microbiology System against *Staphylococcus* species.
Methods: One hundred and ninety five staphylococcal strains (103 *S. aureus*, 42 *S. epidermidis* and 50 other coagulase-negative *Staphylococcus* species) of clinical or stock origin were evaluated in Phoenix and the NCCLS-recommended standard broth microdilution (SBM) reference method. Eighty-eight methicillin-resistant *Staphylococcus* species were included in the test set. Inocula were prepared simultaneously for each test strain in Phoenix and the SBM using an inoculum density equivalent to the NCCLS-recommended 5×10^5 cfu/mL. Dilution ranges for each antimicrobic tested were equivalent in the two systems. Once inoculated, all Phoenix panels were incubated and read automatically in the Phoenix instrument. SBM panels were incubated in ambient air at 35°C for 18–20 h and read manually. Interpretive breakpoints used to determine categorical agreement (CA) were

provided by NCCLS for LZD and ETP, CA-SFM for TEL and the individual drug manufacturer for DAP, GEM and GRN.
Results: Essential agreement of the Phoenix and reference results overall was equal to or greater than 98% for DAP, GRN, GEM and ETP, 94% for LZD and 88% for TEL. CA was between 99 and 100% for LZD, DAP and ETP, and between 92 and 96% for GEM, GRN and TEL. No very major errors (VME) between Phoenix and the reference system were detected with the six antimicrobics tested. Major errors (ME) were encountered only with GEM (two isolates) and TEL (four isolates).
Conclusions: The Phoenix System is effective for use in determining accurate in vitro susceptibility results for the new antimicrobics LZD, DAP, GRN, GEM, ETP and TEL with staphylococci.

P1294 The in vitro analysis of a model antimicrobial system targeted to MRSA

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Objectives: The design, production and preliminary in vitro microbiologic analysis of a Bacterial Inhibitory Protein System (BIP System) with narrow spectrum antimicrobial activity.
Methods and results: A model antimicrobial system has been developed which allows specific targeting to a wide range of individual bacterial species: a gene encoding a Small Acid-Soluble Spore Protein (SASP) is delivered to chosen bacterial cells by means of a modified bacteriophage vector. SASP expressed within vegetative bacterial cells demonstrates a strong antibacterial effect. SASP binds in a nonsequence specific manner to bacterial DNA and alters its conformation from the normal B-like to A-like. This results in inhibition of DNA replication and to some extent transcription. The utilization of bacteriophages as delivery vectors gives the system specificity and a model BIP System has been developed to specifically target *Staphylococcus aureus*, including MRSA. An *S. aureus*-specific bacteriophage has been modified so that its genome contains a SASP gene (sspC) in place of the native phage holin gene. The absence of the holin gene results in phage that are prevented from lysing the infected bacterial cells. This has a beneficial impact by inhibiting the release of inflammatory bacterial cell wall components and reducing the bioavailability to other bacteria of free bacterial DNA (chromosomal, plasmid or transposon) which may contain toxin and/or antibiotic resistance genes. The modified *S. aureus* phage (designated HHBC-11) has been used in in vitro susceptibility studies whereby a range of *S. aureus* (including MRSA) strains have been infected with HHBC-11 and bacterial cell viability monitored. Addition of HHBC-11 phage (1010 pfu) to *S. aureus* cultures ranging from 103 to 108 cfu/mL results in up to a 6-log drop in bacterial viability within 30 min.
Conclusion: The BIP System is a promising antimicrobial agent that can be targeted to specific bacterial species, including *S. aureus* and, in particular, MRSA strains.

Streptococci and Gram-positive bacteria

P1295 Susceptibility to glycopeptides of multiresistant coryneform bacteria

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Introduction: Coryneform bacteria are the heterogenic group of Gram-positive rods, which comprises 15 genera. Many species, mostly from *Corynebacterium* genus belong to the human natural skin flora and mucous membranes. More and more often coryneform are isolated from clinical specimens and examined as etiologic factors of hospital infections.
Objectives: To assess the susceptibility of clinical isolates of multiresistant coryneform bacteria to glycopeptides by MIC.
Methods: The subject of the examination were 88 of multiresistant coryneform bacteria strains. The strains were identified with the help of API Coryne, API ZYM (bioMérieux) and modified of CDC's scheme set. Antibiotic-susceptibility was evaluated according to *E*-test, according to the NCCLS recommendations. *Staphylococcus aureus* ATCC 25923 was used as control strain. Method for comparing two frequencies was used for statistical analysis.
Results: Resistance to many antibiotics was characteristic mainly for *Corynebacterium urealyticum*, *C. amycolatum*, *C. afermentans* ssp. lipophilum species which were isolated species which were isolated from urine (37.2%), wound swabs (30.2%) and drain (16.2%) from patients treated in surgery unit (30.2%), urology unit (22.1%), and intensive care unit (16.3%).

MIC range 0.125–0.50 mg/mL of vancomycin – 14 strains, of teicoplanin – 20 strains.
MIC range 0.75 mg/mL of vancomycin – 42 strains, of teicoplanin – 32 strains.
MIC range 1.00 mg/mL of vancomycin and teicoplanin – 30 strains.
MIC range 1.50–2.00 mg/mL of vancomycin – 2 strains, of teicoplanin – 6 strains.

The differences in percentage of strains with defined MIC values are not statistically significant.
Conclusions: Lipophilic species and *C. amycolatum* strains were in most cases multidrug resistant. All isolated strains were susceptible to glycopeptides, however, it was noticed higher percentage of strains with MICs of glycopeptides in range 0.75–1.00 mg/mL.

P1296 Effect of inoculum conditions on antimicrobial susceptibility testing of *Bacillus anthracis*

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Objective: Anthrax is a zoonotic infectious disease. Antimicrobial therapy and prophylaxis have become important issues because of recent world events. The purpose of this study was to confirm the results of NCCLS antimicrobial susceptibility testing (AST) methods for *B. anthracis* and to further study issues around inoculum conditions that might affect AST results.
Methods: Twenty-five strains of *B. anthracis* isolated from animals from 1964 to 2001 were tested. There were 18 isolates from cattle, 4 from bison and one each from moose, black bear and a goat hide. *B. anthracis* ATCC 4229 was also used. The strains were tested from direct and logarithmic phase suspensions. AST was performed in cation-adjusted Mueller-Hinton (MH) broth or MH agar. The tests were incubated for 18–24 h at 35 °C in ambient air. Spore counts and vegetative cells were estimated by heat shocking for 30 min at 60 °C and performing colony counts. Six of these strains were then tested at different inoculum concentrations (0.5 and 2.0 McFarland standards), and by growing the primary inocula in 0.25% Tween 80, 0.25% Triton X-100 and by disrupting clumps of cells with glass beads prior to susceptibility testing. The agents tested were amoxicillin, amoxicillin-clavulanate, penicillin, meropenem, clarithromycin, doxycycline, tetracycline, ciprofloxacin and levofloxacin.
Results: All 25 strains were susceptible to each of the agents. The range of MICs was marginally narrower with the direct suspension tests. The MIC90 of the log phase cultures were one dilution higher than the direct suspension tests. Disk zone sizes were comparable and consistent with susceptible strains of *Bacillus* species. The percentage of spores in the inocula of 7 strains tested varied from 0 to 15%. MICs and disk zones did not differ when the concentration of organisms was increased from a 0.5 to a 2.0 McFarland, and preparing the inoculum in Tween 80 or vortexing with glass beads did not alter MICs. Triton X-100 was inhibitory.
Conclusions: These strains of *B. anthracis* isolated over a long period of time remain highly susceptible to the antimicrobial agents tested. Despite the fact that this species readily form chains and clumps in various media, the AST

method is robust. There was no quantitative difference in MIC or disk susceptibility with several inoculum variations. This data supports the findings of the NCCLS method development group for broth dilution MICs.

P1297 The susceptibility of *B. anthracis* to 18 different antibiotics

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Objectives: To determine the antimicrobial activity (MIC) and the rate of kill of 18 different antibiotics belonging to different antibiotic groups on two strains of *B. anthracis*.
Methods: Determination of minimum inhibitory concentrations (MICs): *B. anthracis* (Sterne & STI strains) were grown on brain heart agar (BHA) at 370 °C for 24 h. One colony was inoculated into brain heart infusion broth (BHI-B) and was grown overnight at 37 °C. 10 µL containing 105 CFU were added to two-fold dilution of various antibiotics diluted in BHI-B in wells of flat bottom microtiter plates. Growth inhibition was determined following 18 h incubation at 37 °C in ambient air. The MICs were recorded as the lowest concentration of an antibiotic that inhibited visible bacterial growth. Determination of bacterial kill: an overnight culture of *B. antharacis* was diluted 1:1000 with BHI-B in final volumes of 2 mL. Antibiotic solutions in concentrations of ×5 MICs and ×10 MICs (of each strain tested) were added and incubated at 370 °C, samples were obtained at: 0, 0.5, 2, 4, 6, 10, 12 and 24 h for bacterial count.
Results: The fluoroquinolones are ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin. The beta-lactams: penicillin G and amoxicillin. The macrolides clarithromycin and erythromycin. The ketolide telithromycin. Clindamycin, rifampin and quinupristin-dalfopristin (Q/D) had MICs in the range of 0.03–0.25 µg/mL. Ciprofloxacin and penicillin G being the most active with a MIC of 0.03 µg/mL. Erythromycin, vancomycin and the oxazolidinone linezolid were less active (MIC 0.5–2.5 µg/mL). Ceftriaxone was the least active having a MIC of 9.8 µg/mL and chloramphenicol was inactive MIC > 250 µg/mL. Moxifloxacin, Q/D and rifampin showed the most rapid bacterial killing achieving a complete kill (4 log) within 2–4 h at both concentrations. The beta-lactams and vancomycin, demonstrated a 2–4 log reduction within 4–6 h. Ceftriaxone had a similar effect to penicillin G and to amoxycillin. The macrolides, tetracyclines and linezolid demonstrated a slower kill rate while chloramphenicol failed to kill.
Conclusions: The data expands the spectrum of agents presently recommended for the treatment of anthrax (ciprofloxacin, penicillin G and tetracyclines) and adds new options such as other fluoroquinolones, amoxycillin, rifampin and Q/D as potential agents.

P1298 The resistance of Gram-positive bacteria isolated in catheter-associated infections to glycopeptides, in an emergency hospital, Bucharest, Romania, between 2001 and 2002

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Objective: To study the resistance of Gram-positive bacteria to glycopeptides isolated in catheter-associated infections between 2001 and 2002.
Methods: Pathogens were isolated according to Maki method. The resistance to glycopeptides of 152 bacteria isolated from catheter infections was investigated by MIC – 'Microscan Walkaway – Dade Behring', to 6 antibiotics: oxacillin (ox), amikacin (ak), claritromocin (clr), vancomycin (va), teicoplanon (tec), penicillin (p).
Results: The microorganisms most commonly responsible for central venous catheter were *Staphylococcus aureus* 45.35, *S. epidermidis* 20.3%, followed by Gram-negative rods: *Klebsiella neumoniae* 9.2%, *E. coli* 6.5%, *Serratia marcescens* and *Proteus mirabilis* 4.6%, *Pseudomonas aeruginosa* 3.2%, *Citrobacter freundii* 1.9%. *Enterococcus faecium* and *faecalis* were only 2.6 and 1.3%, respectively. For *S. aureus* 71% isolates were resistant to ox (MRSA) 47.4% to ak, 55.4% to cip, 18.5% to clr, 0% to va, 0% to tec. For *S. epidermidis* the resistance was: ox 69.9%, ak 37.1%, cip 52.5%, va 0%, tec 0%. For *E. faecium:* p 83.3%, cip 50%, va 0%, tec 0%, for *E. faecalis*: p 5%, cip 100%, va 0%, tec 0%.
Conclusions: In contrast to another study *S. aureus* was the most frequent isolated. A high incidence of MRSA and MRSE was observed. A good susceptibility to glycopeptides was noted also.

P1299 An in vitro model to evaluate continuous antibiotic lock technique to sterilize ports

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Objectives: Surgically placed venous access ports are used to facilitate long-term intravenous therapy. Bacteria that adhere to these implanted medical devices can become the cause of persistent infections. Treatment of such infections is often difficult due to the existing biofilms on the port inner surface; in this biofilms, bacteria are less vulnerable to antimicrobial agents. A port-associated infection in vitro model was designed to evaluate the efficacy of 16 antibiotic solutions (potentially useful for the antibiotic lock therapy in vivo) to reduce or eliminate intraluminal *S. aureus* port colonization.
Methods: A clinical isolate of a slime-producing *Staphylococcus aureus* was used. Two different concentrations (2 and 10 mg/mL) of vancomycin, cefazolin, fosfomycin and teicoplanin were used alone or in combination with heparin (V2, C2, F2, T2, V10, C10, F10, T10, VH2, CH2, FH2, TH2, VH10, CH10, FH10 and TH10 lock, respectively). Twenty-four hours *S. aureus* biofilms were developed inside the port by static incubation. Then, the ports were drained and filled with the different lock solutions simulating clinical conditions. After different incubation times (1, 3, 5, 7 and 10 days) ports were drained, filled with fresh broth and sonicated. Several dilutions of the final solutions were collected and inoculated on blood agar.
Results: We observed higher reductions in bacterial counts with cefazolin–heparin, fosfomycin–heparin or teicoplanin–heparin locks than with cefazolin, fosfomycin or teicoplanin locks; however, vancomycin–heparin locks appeared to be less effective than vancomycin locks. Catheter intraluminal colonization was eliminated after instillation of V10 as early as day 3. Colonization was eliminated from the port inner surface by day 5 after being treated with FH10, T10 or TH10, by day 7 after being treated with V2, CH2, C10, CH10, T2 or TH2, and by day 10 with VH2, C2 or F10. F2 and FH2 failed to sterilize the port by day 10.
Conclusions: Results of this study demonstrate that prolonged contact with a concentrated antibiotic solution within the port lumen may be effective in eliminating slime-producing *S. aureus*. Cefazolin and fosfomycin were shown to be as effective in removing surface colonization as the most costly glycopeptides. There may be stability problems with vancomycin if heparin is added to the solution locks.

P1300 Resistance and in vitro activity of newer antibiotic agents against Viridans group streptococci blood stream isolates from patients with immunosuppression and endocarditis

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Objectives: Viridans group streptococci (VGS) are a frequent cause of bacterial endocarditis and bacteremia with associated multiorgan failure in patients with neutropenia requiring hospital admittance and intravenous treatment. This analysis examines the in vitro activity of established and new antimicrobials against VGS blood stream isolates.
Methods: During the years 1998–2000 43 blood stream isolates (in 2 blood cultures) were collected at the University Hospital of Vienna, 18 from patients with endocarditis and 26 from immunosuppressed patients. The MICs were tested using NCCLS standards.
Results: MIC50 (MIC range) for penicillin, gentamicin, vancomycin, teicoplanin, amoxicillin/clavulanic acid, quinopristin/dalfupristin, moxifloxacin and linezolid were 0.06 (0.001–1), 4 (0.5–32), 0.5 (0.25–2), 0.06 (0.001–0.25), 0.06 (0.06–0.25), 1 (0.25–4), 0.25 (0.001–1) and 1 (0.06–2), respectively. Drug interaction studies showed indifference for the combinations of vancomycin plus gentamicin, moxifloxacin plus gentamicin and linezolid plus gentamicin. Additionally, MICs were performed in a static biofilm model. In strains which formed a biofilm, the MIC of vancomycin increased 2 times compared with the planctonic MIC whereas MICs of linezolid and moxifloxacin were unchanged.
Conclusion: The in vitro efficacy and low resistance rates of the newer antibiotics linezolid and moxifloxacin suggest their use in a sequential oral treatment of severe VGS infections like endocarditis to shorten hospitalization and thus reduce hospital costs.

P1301 Susceptibility to penicillin, amoxicillin, erythromycin, clindamycin, telithromycin, ofloxacin, levofloxacin and moxifloxacin in recent Belgian viridans streptococci, isolated from the upper respiratory tract

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Objectives: Because resistance to commonly used antimicrobials is increasing in the viridans group streptococci (VGS), we studied the in vitro activities of β-lactam antibiotics, macrolides, ketolides and fluoroquinolones against a collection of recent strains, isolated from the upper respiratory tract.
Methods: VGS were isolated from 92 consecutive throat and sputa samples. MIC testing was performed by *E*-test strips on Mueller–Hinton agar supplemented with 5% sheep blood. Plates were incubated for 24 h, at 37 °C, in CO_2. *S. pneumoniae* ATCC 49619 was used as control strain. NCCLS MIC interpretive standards were used for penicillin (PEN), ofloxacin (OFLO), levofloxacin (LEVO), erythromycin (ERY) and clindamycin (CLI). For moxifloxacin (MOXY) and amoxicillin (AMO) we followed the *S. pneumoniae* criteria, for telithromycin (TEL) the breakpoint recommendations of the manufacturer.
Results: The range, the MIC50, the MIC90 and the percentage of resistant (R), intermediate resistant (I) and susceptible (S) strains are noted in the table.

	Range (mg/L)	MIC50 (mg/L)	MIC90 (mg/L)	R (%)	I (%)	S (%)
PEN	0.012 to >32	0.16	32	23.9	25.0	51.1
AMO	<0.016–48	0.16	8	16.3	7.6	76.1
ERY	<0.016 to >256	3	256	67.4	0.0	32.6
CLI	0.016 to >256	0.064	256	18.5	3.3	78.3
TEL	0.004–3	0.125	0.75	1.1	4.3	94.6
OFLO	0.38 to >32	1.5	3	1.1	18.5	80.4
LEVO	0.125–8	0.5	1	1.1	0.0	98.9
MOXY	0.032–3	0.094	0.19	0.0	1.1	98.9

24% of the ERY-S strains and 61% of the ERY-R strains were penicillin nonsusceptible. 27% of the ERY-R strains showed resistance to CLI. Only 21% of all strains were S to all tested antimicrobials. In both the ERY-R and the ERY-S group the MIC50 (and MIC90) of TEL were lower than the MIC50 (and MIC90) of ERY; in the ERY-R group, the MIC50 for TEL and ERY were, respectively, 0.25 and 6, in the ERY-S group 0.016 and 0.032.
Conclusion: The new ketolide, TEL, and fluoroquinolones, especially LEVO and MOXY, have a good in vitro activity. The MIC90 of LEVO however, is already 1 mg/L. Only 50% of the strains is susceptible to PEN. AMO has a better activity than PEN. The susceptibility rate of ERY is very low. The higher susceptibility to CLI suggest the macrolide resistance is mainly due to an efflux mechanism.

P1302 In vitro susceptibility of viridans streptococci isolated from oral and maxillofacial infections

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Objectives: To investigate the antimicrobial susceptibility of 100 strains of viridans streptococci isolated from pus samples collected from Romanian patients with oral and maxillofacial (OMF) infections. These streptococci strains were found either in association with other bacteria or in pure culture.
Methods: The isolates were identified at species level using the Rapid ID 32 STREP system (bioMérieux). The standardized disk diffusion method (performed with BBL Sensi-Discs, Becton Dickinson) and the *E*-test (AB Biodisk) were used for susceptibility testing of the isolates to: penicillin G (PG), ampicillin (AM), cefotaxime (CT), erythromycin (EM) and clindamycin (CM).
Results: The isolates were identified as: *S. oralis* (58 strains), *S. anginosus* (31 strains), *S. mitis* (4 strains), *S. sanguis* (4 strains) and *S. constellatus* (3 strains). Both antimicrobial susceptibility techniques assigned similar results regarding the susceptibility of the strains. The MICs (mg/L) ranges were: PG 0.008–0.75 (86% Susceptible, 14% Intermediate), AM 0.016–0.5 (98% Susceptible, 2% Intermediate), CT 0.023–0.38 (100% Susceptible), EM 0.016–4 (91% Susceptible, 2% Intermediate and 7% Resistant) and CM 0.016–0.047 (100% Susceptible). The isolates with intermediate susceptibility to PG or to both PG and AM belonged to the species: *S. sanguis*, *S. mitis* and *S. oralis*. Resistance to EM was found among all species except for *S. constellatus*.
Conclusions: (1) Reduced susceptibility to beta-lactam antibiotics was detected only among the mitis group isolates. (2) Resistance to EM was less than 10%, but CM was fully active. (3) The results recommend CM as an alternative for antimicrobial therapy of OMF infections, which are usually mixed infections, involving strictly anaerobic bacteria (frequently beta-lactamase producers) and aerobic microorganisms, especially oral streptococci.

P1303 Group B *Streptococcus* in a tertiary hospital, Greece (1998–2002)

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Objectives: To detect the isolation rate and the sensitivity of Group B *Streptococcus* (GBS) to common antibiotics during a period of 4 years (1998–2002).
Methods: A total number of 890 vaginal secretions of non-pregnant women, 478 prostatic fluids, 1886 bronchial secretions, 2392 pus mainly from decubitus ulcers, 23 982 urines were cultured in appropriate media. The susceptibility to antibiotics were tested by microdilution method according to NCCLS recommendations. The strains *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923 were used as controls.
Results: GBS was isolated from 5.2% vaginal secretions, 1.4% pus (one from bartholinian abscess), 1.5% prostatic fluids, 0.63% bronchial secretions and 0.36% urines. Seventeen patients (19.5%) had clinical evidence of urinary tract infections (UTI), three had prostatitis, one had bartholinitis with diabetes mellitus as underlying disease. In all cases GBS was concomitant isolate with other microorganisms in vaginal secretions and pus. All strains were sensitive to penicillin, erythromycin, clindamycin, vancomycin, ciprofloxacin and linezolid.
Conclusions: (1) GBS seems to be a potential uropathogenic microorganism. (2) GBS contribute to decubitus ulcers and represent potential pathogen in invasive GBS diseases. (3) GBS remain sensitive to the most common antibacterial agents used in Gram-positive infections.

P1304 Infections and antimicrobial susceptibility of *Streptococcus* group B in nonpregnant adults

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Background: Group B streptococci (GBS) are an important cause of neonatal sepsis and meningitis, and maternal infection. GBS are also responsible for infections in nonpregnant adults.
Objectives: To survey the GBS infections and the registration of GBS susceptibility to antimicrobial agents in nonpregnant adults.
Methods: 99 GBS strains were isolated from adults' infections during the period 01/01/2001–30/12/2002. The identification was made by conventional methods (the detection of B Ag according to Lancefield was made by slidex strepto kit – bioMérieux) and the antimicrobial susceptibility was determined by the breakpoint system: mini API (bioMérieux).
Results: 99 GBS strains were isolated from 99 patients (88 women and 11 men). Fifty-two patients were treated as inpatients and 47 as outpatients. Classification of isolates by specimen type and sex was: urine 51 (43 women and 8 men), blood 1 (man), vaginal fluid 42 (women) and wound infections 5 (3 women and 2 men). Wound infections were: 3 diabetic foot and 2 burns. In one case (1 man) GBS was isolated from diabetic foot and blood culture simultaneously. The susceptibility of GBS strains to the antimicrobial agents was: 85.8% to penicillin, 96% to vancomycin, 91.9% to ampicillin, 86.9% to cefuroxime, 77.7% to ciprofloxacin, 68.7% to clindamycin, 65.6% to erythromycin, 52.5% to trimethoprim-sulfamethoxazole, and 33.3% to tetracyclines.
Conclusions: GBS was found to be the causative agent to considerable number of urinary tract infections mainly in women. Ampicillin proved to be the most active antimicrobial agent against GBS in vitro.

P1305 In vitro activity of 7 antimicrobial agents against *Streptococcus agalactiae* clinical isolates

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Objective: The aim of this study was to evaluate the in vitro activity of penicillin, cefotaxime, vancomycin, linezolid, erythromycin, clindamycin and moxifloxacin against *Streptococcus agalactiae* isolated from clinical samples in our Hospital.
Materials and methods: The following 32 samples were studied: 21 (65.6%) from female genital tract infection, 6 (18.7%) from patients with urethritis, 4 (12.5%) from infected wounds and 1 (3.1%) from blood culture. Antimicrobial activity was determined by an agar dilution method using 5% blood plates, according to NCCLS recommendations. ATCC-29213 *Staphylococcus aureus* was used as control strain.
Results: The following MIC values were obtained for all organisms tested: MIC50 and MIC90 were 0.03 and 0.12 mg/L for penicillin, 0.016 and 4 mg/L for erythromycin, 0.5 and 0.5 mg/L for vancomycin, 1 and 1 mg/L for linezolid, 0.03 and 4 mg/L for clindamycin, 0.06 and 0.12 mg/L for clindamycin, 0.06 and 0.12 mg/L for moxifloxacin and 0.03 and 0.12 mg/L for cefotaxime. All strains showed susceptibility to penicillin, cefotaxime, vancomycin and linezolid. 28.5% were resistant to erythromycin and 26.1% resistant to clindamycin. Not susceptibility breakpoints exists for moxifloxacin, however, all strains were inhibited at a concentration of 0.12 mg/L or lower.
Conclusions: In our study, most strains showed susceptibility to classical antibiotics. Linezolid could be a good alternative to vancomycin in treatment of infections due to *S. agalactiae*. Moxifloxacin showed a very good activity against the strains tested.

P1306 Characterization of antimicrobial susceptibility patterns among community-acquired isolates of beta-hemolytic streptococci in Latin America: results from the SENTRY Antimicrobial Surveillance Program (2001)

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Objectives: Recently, beta-hemolytic streptrococci (BHS) have been recognized as an increasing cause of sepsis and soft tissue infections in adults with chronic illnesses. The main objective of this study was to determine the level of emerging resistances (R) among BHS isolates collected in the LA region monitored by the SENTRY Program.
Methods: A total of 198 BHS clinical specimens were collected from patients with community-acquired infections in 10 LA sites between January and March 2001. The susceptibility (S) to penicillin (PEN) and other antimicrobials was evaluated by broth microdilution method (NCCLS). Quality control was performed using ATCC strains.
Results: PEN (MIC50, 0.015 mg/L) was the most potent agent with only one isolate of group A streptococci from a Chilean site having slightly reduced susceptibility (0.25 mg/L). Although azithromycin (MIC50, 0.06 mg/L) had shown to be the second most potent drug against GBS, only 88.9% of isolates were S to this agent. Interestingly, the R phenotype of MLSB was present in less than 3% of BHS isolates. Quinupristin/dalfopristin, linezolid, vancomycin, and gatifloxacin inhibited the growth of all GBS isolates at concentrations of $\leq$1, 2, 1, and 0.5 mg/L, respectively. Tetracycline showed the lowest S rate (53.5%) with greatest R among group B streptococci.
Conclusions: Our results indicated that BHS isolates were highly susceptible to beta-lactam antimicrobial agents. It confirms that PEN remains the best therapeutic option for treatment of BHS infections in Latin America. However, continuing surveillance will be warranted to detect any decrease in the susceptibility such as those noted for macrolides.

P1307 Rising penicillin minimum inhibitory concentration (MIC) – a cause for concern in group A beta-hemolytic streptococci

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Objectives: (a) Evaluate the occurrence of recurrent bacterial pharyngitis caused by Group A beta-hemolytic streptococci (GABHS). (b) Study the incidence of macrolide resistance in these isolates. (c) Identify the type of macrolide resistance. (d) Detect the minimum inhibitory concentration to penicillin in these isolates.
Material and methods: Eighty-eight patients of recurrent pharyngitis attending the ENT clinics formed the Medical group and 28 patients undergoing adenoido-tonsillectomy formed the Surgical group. Twenty normal healthy volunteers formed the Control group. Two to four throat swabs were collected from each patient and the control subjects. In addition, tonsils/adenoids recovered at surgery were studied. The throat swabs, tonsils and adenoids recovered from patients were subjected to microscopy, culture, biochemical tests and confirmation using group specific antisera. Antimicrobial susceptibility testing was performed by disc diffusion using Kirby Bauer method. The minimum inhibitory concentration (MIC) was done using E-strips for penicillin and erythromycin. Double disc diffusion test was performed with clindamycin and erythromycin to detect the type of macrolide resistance.
Results: A total of 108 throat swabs and 28 tonsils were studied. Twenty percent of the patients in the medical group and 23% of the patients in the surgical category had GABHS. None of the samples from the control group grew GABHS. Macrolide resistance was observed in 30% of the isolates by the Kirby Bauer method and 16.7% of them showed resistance to erythromycin by E-strip test. The MIC values for penicillin ranged from 0.032 to 0.250 μg/mL.
Conclusions: (a) Macrolide resistance is found to be higher than in previous reports. (b) The type of resistance seen in our strains is the M type. (c) Increasing levels of penicillin MIC were observed in the streptococci with a strain having MIC value outside the sensitive range as given by the NCCLS (0.250 μg/mL). As with *Streptococcus pneumoniae*, GABHS is also showing a rising trend in terms of macrolide resistance. The MIC values for penicillin are also edging towards the intermediate levels.

P1308 Patterns and phenotypes of resistance in streptococci

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Introduction: Three mechanisms of resistance to macrolides are actually known: target modification, inactivation, and drug efflux. The target modification, occurring in the ribosome, through the production of methylase codified by the gene erm, leads to the development of resistance to the majority of macrolides, lincosamides and streptogramines B (MLSB resistance). While the resistance by inactivation was never observed in streptococci, the mechanism of active reflux was described in *S. pyogenes* and less frequently in *S. pneumoniae* as leading to the phenotype M (resistance to macrolides, but susceptible to lincosamides and streptogramines B).
Objective: The objective of this study was to analyze the phenotype of resistance to in *S. pyogenes*, *S. pneumoniae* and *S. milleri* of all the isolates at the Saint George University Hospital.
Material and methods: A total number of 195 isolates (including 65 *S. pyogenes*, 76 *S. pneumoniae*, and 54 *S. milleri*) coming from the clinical specimens of the Saint George University Hospital – SGH Laboratory between 1-1-2000 and 1-1-2002 were included in the study. The identification and antibiotic susceptibility testing were done as recommended by the NCCLS. Phenotypic characterization of resistance to erythromycin was realized using the double disc method described by Sepala and cols; the phenotypes MLSB (I and C) and M were differentiated based on the diameters of inhibition as well as the presence of blunting around the clindamycin and erythromycin discs.
Results and discussion: In our study, no resistance to penicillin was found in *S. pyogenes* or *S. milleri*. 47.4% of the isolates of *S. pneumococcus* were resistant to penicillin. This finding is consistent with data coming from EARSS 2001 where ''the highest average of penicillin nonsusceptibility in *Pneumococcus* is found for the Mediterranean countries. Resistance in general to Erythro and Clinda varied between 7 and 24%. As per the phenotypes of resistance, 46.7% of *S. pyogenes* and 20% of *S. pneumoniae* showed the phenotype M. This phenotype was detected in 2 isolates (out of 6) of *S. milleri* resistant to erythro, the majority showed the Phenotype MLS. Table 1 shoes the percentages of resistance to the different antibiotics used.

Table 1

	S. pyogens Nb (%R)	*S. pneumoniae* Nb (%R)	*S. milleri* Nb (%R)
Penicillin	0 (0)	36 (47.4)	0 (0)
Erythromycin	15 (23.1)	10 (13.2)	6 (11.1)
Clindamycin	8 (12.3)	8 (10.5)	4 (7.4)

Enterococci

P1309 Linezolid susceptibility of glycopeptide-intermediately susceptible *Staphylococcus aureus* (GISA) – the Dutch experience

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Introduction: The occurrence of glycopeptide-intermediate-susceptible *Staphylococcus aureus* (GISA) warrants alternative treatment. One of the alternative antimicrobial agents used to treat GISA infections is linezolid, a new oxazolidinone.
Objective: Aim of the study was to assess the intra- and interlaboratory reproducibility of the *E*-test macromethod for testing linezolid.
Methods: At total of 100 isolates (10 MRSA, 9 hGISA and 6 GISA – as determined by PAP-AUC ratio, each strain in quadruplets) were sent to 8 Dutch study centers. Investigators were totally unaware as to the isolates's phenotype. All strains were PFGE typed to eliminate clonal duplication. *E*-test MICs were determined using an inoculum density of 0.5 McFarland turbidity. 200 mL of the inoculum suspension was delivered onto a 90-mm MH agar plate and streaked out evenly. After drying *E*-test strips (AB BIODISK, Solna, Sweden) for linezolid (0.016–256 mg/mL) were applied, all plates were incubated at 35°C for 24 h, and read by two independent technicians. Three laboratories read the plates at 80% inhibition (as recommended by the company) others at 100% as recommended for, e.g. glycopeptides.
Results: The results are shown in the table.

		MIC low		MIC high		Average MIC		Average range	
	N	100%	80%	100%	80%	100%	80%	100%	80%
MRSA	10	1.35	0.44	2.8	2	2.31	1.38	0.56	0.64
hGISA	9	0.8	0.5	2.2	1375	1.4	0.88	0.27	0.27
GISA	6	0.8	0.5	1.7	1.25	1.38	0.88	0.28	0.35

Conclusions: Linezolid MICs ranged between 0.8 and 2.8 mg/L and 0.44–2 mg/L when read at 100 and 80% inhibition, respectively. Surprisingly, MICs were generally lower in GISA/hGISA strains than in glycopeptide-susceptible MRSA strains. Furthermore, the average range when comparing the inter-laboratory reproducibility was wider in the MRSA strains and in those centers reading at 80% inhibition. The difference was less evident when testing hGISA/GISA. The intralaboratory reproducibility was generally excellent. All strains were considered being susceptible according to the prescribing information.

P1310 Comparison of linezolid and daptomycin activity against resistant Gram-positive bacterial isolates in cancer patients

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Objectives: The increasing incidence of multidrug resistant Gram-positive (G+) bacterial infections provides a significant challenge in treating immunocompromised hosts, with few therapeutic options. The activity of linezolid (LZD) and daptomycin (DAP), an investigational lipopeptide, was evaluated against clinical G+ isolates in cancer patients.
Methods: Single blood isolates from hospitalized cancer patients were collected; LZD and DAP MICs were determined in duplicate by macrobroth dilution according to NCCLS guidelines. Calcium supplementation of Mueller-Hinton broth was used for DAP to achieve a final concentration of 50 mg/L.
Results: For all G+ isolates ($N = 257$), the MIC50,90 (range) for LZD was 2.4 (0.125–64) mg/mL and 1,4 (0.03–8) mg/mL for DAP, respectively. Of 119 *Enterococcus*, the LZD and DAP MIC50, 90 (range) was: for 26 *E. casseliflavus* (24 VRE) 2.4(1–4) and 4.8(4–8); 52 *E. faecium* (48 VRE) 2.4 (0.5–4) and 2.4 (1–8); 24 *E. faecalis* (2 VRE) 4.4 (2–64) and 2.4 (0.5–4). The LZD and DAP MIC50, 90 (range) against 128 *Staphylococcus* was 2.4 (1–8) and 0.5.1 (0.25–2), comprised of 29 MSSA, 8 MRSA, 12 MSSE, and 59 MRSE. DAP retained activity against 3 organisms with reduced LZD susceptibility (one MSSA, and two VS *E. faecalis*, LZD MIC = 8, 32, 64; DAP MIC = 1, 1, 2).
Conclusions: LZD and DAP demonstrated activity against many of the MDR G+ bacteria that frequently cause infections in cancer pts., however, several LZD-resistant isolates were identified. LZD and DAP may have a potential role for in the treatment of infections in cancer patients, including febrile neutropenia.

P1311 In vitro antimicrobial susceptibility of linezolid against Gram-positive cocci in suspensions, biofilms and resuspended biofilms

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Objective: Diagnostic microbiology laboratories test the susceptibilities of bacteria in suspension (planktonic bacteria). However, infections related to indwelling medical devices are difficult to treat due to the biofilm mode of bacterial growth. Biofilms often dislodge and enter the bloodstream, causing systemic infections. The objective was to compare the invitro activity of linezolid against bacteria in suspension, in biofilm and in dislodged biofilm modes of growth.
Methods: We tested the susceptibilities of linezolid, against 77 clinical isolates of MRSA, 43 MSSA, and 46 *S. epidermidis* using a broth microdilution technique (NCCLS M7-A5). The MIC90 for linezolid against MSSA, MRSA, and *S. epidermidis* in was 4, 1, and 1 μg/mL, respectively. To form biofilms the organisms were grown in 96-well microtiter plates by culturing $1–2 \times 10^6$ cfu/mL in 200 mL of TSB for 24 h. Plates were then washed twice with PBS and 100 mL of TSB was added. Duplicate plates were used to dislodge the adherent growth in order to form resuspended (dislodged) biofilms. 100 mL of the antimicrobial agent were added at concentrations of MIC90, 50, 500, and 1000 μg/mL. The microtiter plates were incubated for 24 h to allow interaction between the antimicrobial agents and the bacteria. Colony counts on serial dilutions before and after the addition of linezolid were used to calculate percent viability.
Results: The results are shown in Table 1.

Table 1

			% viability							
			Biofilm				Resuspended biofilm			
Organism	MIC_{90} (μg/mL)	MBC_{90} (μg/mL)	MIC_{90}	50 μg/mL	500 μg/mL	1000 μg/mL	MIC_{90}	50 μg/mL	500 μg/mL	1000 μg/mL
S. aureus (MSSA)	4	>64	98.5	38.7	37.2	37.1	89.1	29.1	32.2	36.8
S. aureus (MRSA)	1	>64	96.0	18.4	5.1	4.7	89.9	4.9	2.9	4.72
S. epidermidis	1	32	83.9	46.7	31.8	43.3	77.9	41.1	23.7	42.6

Conclusion: Our conclusions are that routine susceptibility testing on bacterial suspensions (planktonic mode of growth) does not predict the susceptibilities of bacteria in the biofilm (sessile mode of growth).

P1312 In vitro activity of glycopeptides/erythromycin resistant *Enterococcus faecium* to linezolid from a Greek tertiary hospital (1998–2002)

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Objectives: The aim of this study was to examine the sensitivity of the new antimicrobial drug linezolid (L) against glycopeptides/erythromycin resistant *E. faecium* isolates during 1998–2002.
Material and methods: Forty-six glycopeptides/erythromycin resistant strains isolated from urine: 16, pus: 15, blood: 4, catheters: 2, bronchial secretions: 4 and stool: 5 were examined. They belonged to patients admitted to ICU; 29, Surgical department: 10 and Internal Medical Department: 7. All strains carried the *vanA* and *ermB* genes without lactamase production and

exhibited 13 distinct chromosomal macrorestriction genotypes. The sensitivity to (L) was tested by microdilution method according to NCCLS recommendations and *E*-test (AB BIODISK, Solna Sweden) according to the manufacture's instructions.
Results: Linezolid MIC 50 – >1.5 μg/mL, MIC 90 – >2 μg/mL (range 0.25 – >32 μg/mL).
Conclusion: Linezolid seems to provide a remarkable advantage in the management of glycopeptides/erythromycin resistant *E. faecium* infections. Resistance to linezolid however, has been observed, although the new drug is prescribed only the last year in our country.

P1313 In vitro effect of linezolid against vancomycin-resistant enterococci

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Objectives: Vancomycin-resistant enterococci (VRE) have emerged as important pathogens. The reported incidence of infections with VRE is increasing dramatically and becoming endemic in many hospitals throughout the world. Although these bacteria are still relatively rare in Turkey, the incidence seems to be increasing. Most VRE are also resistant to multiple other drugs which have been used for treating VRE infections. So treatment options and effective antimicrobial agents for VRE are often limited. Linezolid is the first oxazolidinone that has been introduced to therapy in the early 2001. It has a bacteriostatic effect against gram positive bacteria including VRE. This drug inhibits bacterial protein synthesis via binding to the 50S ribosomal subunit to prevent translation. The drug lacks cross-resistance with other antimicrobials. The aim of this study was to investigate the in vitro susceptibility of VRE strains isolated in our hospital to linezolid, and to compare this with those of to ampicillin, erythromycin, tetracycline and moxifloxacin.
Methods: The study was performed in a 600 bed University Hospital during the period of 8 months in 2001. The enterococci were isolated from rectal swab samples of 163 hospitalized patients and identified to the species level by conventional methods and API 20 Strep System (bioMérieux, France). Isolated 116 enterococci strains were then tested for vancomycin resistance by screening method using BHI agar containing 6 μg/mL vancomycin. This test yielded 13 VRE strains. The susceptibility pattern of the 13 VRE strains against five antibiotics mentioned above were determined by disk diffusion method. Results were interpreted according to the criteria by 'The Swedish Reference Group for Antibiotics' for linezolid, by recommendation of Barry and coworkers for moxifloxacin, and by NCCLS for other antibiotics.
Results: Of the 13 VRE strains (3 were *E. faecalis*, and 10 were *E. faecium*) none was resistant to linezolid, 9 (1 *E. faecalis*, 8 *E. faecium*) were resistant to ampicillin, 8 (2 *E. faecalis*, 6 *E. faecium*) to erythromycin, 9 (2 *E. faecalis*, 7 *E. faecium*) to tetracyclin, and 10 (2 *E. faecalis*, 8 *E. faecium*) to moxifloxacin.
Conclusion: Overall, the in vitro data demonstrated that, linezolid was very active against VRE. So this drug will be a viable alternative in treatment of VRE infections that are becoming increasingly intractable.

P1314 Susceptibility patterns of enterococci causing infections

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Objectives: Enterococci are among the common organisms associated with hospital-acquired infections. The emergence of enterococci as significant pathogens is a matter of concern because these organisms are inherently resistant to a number of antimicrobial agents. In this study, we examined in vitro activities of different antibiotics to 103 enterococcal isolates (derived from bloodstream infection, urinary tract infection, surgical site infection and central nervous system infection between January 2000 and December 2001) collected in the hospital of Istanbul Medical Faculty.
Methods: MICs of penicillin G, ampicillin, gentamicin, ciprofloxacin, ofloxacin, levofloxacin, grepafloxacin, trovafloxacin and gemifloxacin were determined by the NCCLS recommended broth microdilution testing method. MIC of vancomycin was determined by the *E*-test (AB Biodisk, Solna, Sweden) on Brain-Heart Infusion agar (Oxoid, Hampshire, UK). High-level disks of 120 μg gentamicin (Oxoid, Hampshire, UK) and 300 μg streptomycin (Oxoid, Hampshire, UK) were used to screen for high-level aminoglycoside resistance (HLAR). Production of beta-lactamase was tested with nitrocefin-based beta-lactamase test (Oxoid, Hampshire, UK).
Results: Amongst the 103 strains of *Enterococcus* spp. 71 (69%) were identified as *E. faecalis* and 32 (31%) as *E. faecium*. While over 75% of *E. faecium* isolates were resistant to penicillin and ampicillin, approximately 25% of *E. faecalis* isolates were resistant to penicillin and ampicillin. None of the *E. faecalis* and *E. faecium* isolates were resistant to vancomycin. While 17 (52%) of *E. faecium* isolates exhibited high-level gentamicin resistance (HLGR), high level streptomycin resistance (HLSR) was detected in 24 (74%) of the isolates. In contrast HLGR and HLSR rates for *E. faecalis* were 14 (20%) and 22 (31%), respectively. Both HLGR and HLSR were detected with higher frequency in ampicillin resistant isolates. Among quinolones gemifloxacin and trovafloxacin were the most potent antibiotics tested for *E. faecalis* and *E. faecium* isolates followed by grepafloxacin, levofloxacin, ciprofloxacin and ofloxacin. There was no increase in MIC90 values of the quinolones in ampicillin resistant isolates in comparison with ampicillin susceptible isolates.
Conclusion: Our data suggests newer quinolones would be good alternative agents to use especially for combination drug therapy where enterococci with ampicillin resistance and HLAR are prevalent.

P1315 Vancomycin susceptibility still prevails in Norwegian enterococcal bacteremias

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Objectives: The vancomycin-resistant enterococcus (VRE) was first discovered in Europe in 1986 and has from 1995 become endemic in many hospitals in the USA, primarily infecting immunocompromised and severely ill patients. In Europe hospital outbreaks have been controlled, but carriage of VRE is endemic among healthy people and animal husbandry. At present only one hospital outbreak of VRE has occurred in Norway and carriage of VRE among hospitalized patients is near absent, but VRE is found in poultry farms previously exposed to avoparcin, a glycopeptide growth promoter. We wanted to see if any unrecognized VRE could be found among blood culture isolates (BCIs) from a tertiary care hospital (The National Hospital) in Norway, and also test other relevant antimicrobials against these enterococcal isolates.
Methods: BCIs were identified using the Rapid ID 32 STREP (bioMérieux, France). Enterococcal isolates from 11.06.1997 to 15.05.2001 were included and tested against 10 antibiotics using the *E*-test (AB biodisk, Sweden). Norwegian breakpoints were applied. Isolates with vancomycin MIC ≥ 8 mg/L were tested further by polymerase chain reaction (PCR) using primers specific for the *vanA*, *vanB* and *vanC* vancomycin-resistance genes and electrophoresis.
Results: One hundred and eleven enterococcal isolates (6,9% of all BCIs) were included. The species distribution was 68% *E. faecalis*, 23% *E. faecium*, 6% *E. gallinarum*, 1% *E. hirae* and 1% *E. avium*. Among all enterococcal isolates we identified phenotypical resistance against ampicillin in 17% and imipenem in 23% (both essentially due to *E. faecium*), erythromycin in 22%, tetracycline in 55%, linezolid in 0%, quinupristin/dalfopristin in 59% (only *E. faecalis*), vancomycin 0% and teicoplanin in 0%. We found high-level resistance against streptomycin in 33% and against gentamicin in 17% (the latter in *E. faecalis* only). We identified no *vanA* or *vanB* resistance genes among the 17 isolates with MIC for vancomycin ≥8 mg/L, while all *E. gallinarum* contained the *vanC* resistance gene.
Conclusion: We found no VanA or VanB resistance, 6% of isolates contained the intrinsic nontransferable VanC resistance. Quinopristin/dalfopristin is very seldom used in Norway, resistance in *E. faecalis* reflects the already known low activity against this species. The glycopeptides and linezolid are still alternative treatments in Norwegian enterococcal bacteremias when initial treatment with ampicillin and gentamicin fails.

P1316 In vitro activities of seven antimicrobial agents against clinical isolates of enterococci

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Objectives: To evaluate the in vitro activities of seven antimicrobial agents against enterococci, isolated from clinical specimens obtained from hospitalized patients.
Methods: A total of 301 enterococcal isolates (266 *E. faecalis*, 22 *E. faecium* 8 *E. gallinarum*, 1 *E. hirae*, and 1 *E. raffinosus*) were tested. Specimens included in the study were pus swabs (152), blood cultures (18), and urine (131). The MICs of penicillin, ampicillin, vancomycin, gentamicin, streptomycin,

ciprofloxacin, and rifampin were determined by an agar dilution method according to NCCLS recommendations. All isolates exhibiting resistance to penicillin or ampicillin (MIC > 16 mg/L) were examined for beta-lactamase production with the nitrocefin disk test.
Results: The MIC90s for *E. faecalis* isolates were determined to be 4 mg/L for penicillin, 2 mg/L for ampicillin and vancomycin, >2048 mg/L for both aminogyicosides, 1 mg/L for ciprofloxacin, and 8 mg/L for rifampin. *E. faecium* isolates were more resistant to both penicillins and rifampin with MIC90s = 32 mg/L. High-level resistance to gentamicin (>500 mg/L) and streptomycin (>2000 mg/L) was detected in 38.0 and 41.0% of *E. faecalis* isolates, and in 31.8 and 45.4% of *E. faecium* isolates, respectively. All isolates tested were susceptible to vancomycin. Beta-lactamase activity was not detected in two *E. faecalis* and four *E. faecium* isolates with MICs for penicillin of 32 mg/L or 64 mg/L
Conclusions: The results of this study show high level of resistance to penicillins aminoglycosides and rifampin in *E. faecium* isolates. Resistance to penicillin in *E. faecalis* isolates is low.

P1317 Experience with two in vitro methods for detecting antibiotic synergy against streptococcal and enterococcal isolates in patients with infective endocarditis

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Introduction: Combinations of antimicrobial agents, which have been shown to be synergistic in vitro, by the time-kill method have been associated with a favorable clinical outcome in the treatment of enterococcal and viridans streptococcal endocarditis.
Objectives: (i) To assess the performance of the time-kill method against drug combinations known to be synergistic. (ii) To compare the *E*-test method for synergy prediction with the time-kill method.
Methods: Eight isolates from patients with infective endocarditis that met Duke's criteria admitted during 2002 at Johannesburg Hospital were studied. We performed synergy tests using time-kill, and *E*-tests methods with the penicillin and gentamicin combinations on five streptococcal, three enterococcal, and vancomycin and gentamicin on two enterococcal isolates.
Results: Synergy was presented in the table. Three patients (37.5%) died, two without treatment and HIV positive, and a third after 6 weeks of treatment. All three *E. faecalis* isolates were susceptible to penicillin and vancomycin and confirmed with MIC results; one *S. mitis* and one *S. sanguis* were intermediate susceptible (MIC = 1 for both). The time-kill method showed synergy with 9 out of 10 isolates tested with known synergistic combinations. The *E*-test method showed synergy with four isolates, three of which also showed synergy with the time-kill method. The other isolates showed either an indifference or antagonism.

Organism	Drug combination	*E*-test FIC index	Time kill 4 h	Time kill 8 h	B/C
Enterococcus faecalis	Pen-genta	2.27 (A)	S	S	7
	Vanco-enta	1.33 (I)	S	S	
Enterococcus faecalis	Pen-genta	1.25 (I)	S	S	7
	Vanco-genta	1.75 (I)	S	S	
Streptococcus mitiz	Pen-genta	0.065 (S)	S	S	*
Streptococcus sanguis	Pen-genta	0.44 (S)	I	I	3
Enterococcus faecalis	Pen-genta	1.67 (I)	S	S	**
Streptococcus mitiz	Pen-genta	3 (A)	I	S	*
Streptococcus mitiz	Pen-genta	0.058 (S)	I	S	3
Streptococcus.constellatic	Pen-genta	0.07 (S)	I	S	Unknown

S, synergy; I, indifference; A, antagonism; FIC, (fractional inhibitory index): synergy FIC ≤0.5, indifference FIC >1 and <2, antagonism FIC ≥2; B/C, days of negative blood culture; *died without treatment, **died after 6 weeks of treatment.

Conclusions:
(1) The time-kill method performed well in predicting synergy in the case of combinations expected to be synergistic.
(2) The *E*-test correctly predicted synergy with four out of five streptococcal isolates. Indifferent and antagonistic results occurred with the four isolates. Further investigations are required to confirm this trend.

Helminth infection: clinical aspects

P1318 Comparison study between surgical treatment of hydatid and medical treatment with albendazole

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Aim of study: Iraq is an area of heavy infestation with an Echinococcus *Granulosus* and the disease called hydatid cyst is still a major health hazard in this country, so our aim in this study is to compare between right and left lobes involvement from the point of rate of occurrence, clinical presentations, intraoperative findings, post operative complications, effect of albendazole and sex distributions. So we hope by this study to reach for the best method for the diagnosis and treatment.
Patients and methods: Between October 1993 and October1997, 75 patients presented with hydatid disease of the liver were admitted to our general surgical department in medical city teaching hospital. A retrospective study of surgical and medical treated patients was planned. A full history was obtained from every patients emphasizing on certain points including age, sex, occupation, residency, contact with pet animals, family history. Each point was thoroughly examined. All patients were diagnose by ultrasound and few of them by serology. So we compare between the right and left lobes hydatid and comparison with albendazole therapy.
Results: Seventy-five patients with hydatid cyst of liver are surgically treated, 60‰ of patient were females and 40% were males with f/m 1.5:1, the age range from 10 to 60 years, 33.3% were from rural population and the others from urban districts, 78.7% with hydatid of right lobe and 12% in left lobe and 9.3% bilateral, 20% of the patients we gave them albendazole tablet for 6 months compared with those undergoes surgery only, some of them (4%) complain from reoccurrence compared with those taking albendazole therapy in which neither of them reoccurrence.
Conclusion: (1) Hydatid disease of the liver is still very common in Iraq (it is a malignant disease of Iraq). (2) Irradications started from the education of the patient about the disease. (3) Surgery is the treatment of choice, but albendazole therapy has its place in treatment of hydatid especially the disseminated disease and it is used as asynergestic therapy with surgery or is used alone especially in those they have contraindications for surgery.

P1319 Primary renal hydatid cyst

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Objective: A case of an unusual renal hydatid cyst is reported. Detection of antiechinococcal antibodies by indirect hemagglutination (IHA) contributed to the diagnosis.
Material and methods: A 72-year-old man admitted to our hospital because of intense pain in the right renal region and heamaturia. He also mentioned elimination of 'crashed grape like forms' during urination. This material was collected and examined histopathologically. The laboratory analysis included blood examination, abdomen ultrasound, CT scan of chest and abdomen and detection of antiechinococcal antibodies by indirect hemagglutination (IHA).
Results: Laboratory studies showed anemia (Ht = 29%) and leukocytosis (WBC = 17 000/mm^3 with 8% eosinophils). Anti-echinococcal antibodies were presented in a title of 1:1280. Chest X-ray was normal, while the abdomen X-ray revealed a round calcified shadow in the right kidney. Ultrasound examination revealed a cystic formation with peripheral calcification. No other cystic formation were noted. On CT scan a cystic formation without uptake of the sciagraphic was noted. Finally, histologic examination of the eliminated in the urine cysts revealed that the cystic formation was an echinococcal daughter cyst. Successively, right nephrectomy was performed. Intraoperatively there was some difficulty in dissecting the right kidney because of the solid adhesion to the peritoneum. Histologic examination of the kidney confirmed the initial histologic diagnosis.

Conclusions: Renal echinococcosis that represents 4% of all cases of echinococcosis is rare in Greece. The typical elimination of 'crashed grape like forms' after acute urinary pain is almost pathognomonic. Serologic test of IHA is sensitive and specific for the diagnosis of echinococcosis.

P1320 Hydatid cyst of the left ventricle

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Objective: We report a case of isolated hydatid cyst of the left ventricle, an unusual localization, that manifested symptoms of left heart failure. Detection of antiechinococcal antibodies by indirect hemagglutination (IHA) contributed to the diagnosis.
Material and methods: A 41-year-old man admitted to our hospital because of an incident of loosing consciousness. The patient mentioned symptoms of intense dizziness, tachycardia accompanied by arrhythmia, easy fatigue and dyspnea. Laboratory examination included ultrasound and CT scan as well as detection of antiechinococcal antibodies by indirect hemagglutination (IHA).
Results: ECG showed strain of the left ventricle, the chest X-ray showed augmentation of the cardiothoracic index. Cardiac echography revealed dilatation of the left ventricle with elevation of end diastolic diameter (7.8 cm), functional decrease with ejection fraction 41% and a cystic formation, sized 4.9 cm × 5.2 cm, containing daughter cysts. CT scan of the chest confirmed the existence and the size of the cystic formation. Blood analysis revealed anemia, leukocytosis and eosinophilia. Anti-echinococcal antibodies were detected in a title of 1:5120. The cyst was removed surgically. The histological examination confirmed the diagnosis of *Echinococcus granulosus* accompanied by daughter cysts. The patient recovered completely without any sequela.
Conclusions: The localization of the hydatid cyst in the heart is extremely rare (0.5–2%) relatively to the incidence of echinococcosis. Imaging (U/S and CT scan) and immunodiagnostic techniques (IHA and ELISA) contribute to diagnosis of the disease. Surgical treatment results to full recovery. In regions where hydatosis is endemic, cardiologists should be considered the disease in the differential diagnosis of heart failure and thoracic pain, in order to minimize the severity and prevent complications of the disease.

P1321 A recurrence hydatid cyst case after surgical intervention

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Hydatid cyst (echinococcosis), a zoonotic disease commonly seen in some regions of world and Turkey. Musculoskeletal system involvement rarely occurs in hydatid cyst. Surgical removal of hydatid cysts are frequently complicated by cysts rupture, spillage of contents, and secondary recurrences. In this paper a 48-year-old male, whose cyst with vertebral involvement recurred after surgical intervention is presented. Diagnosis was confirmed by histopathological examination of surgical material, radiological imaging and positive serology. Albendazole was started to the patient and continued for 3 months. In conclusion, patients with vertebral lesions of cystic structure should be evaluated by physicians respect of the hydatid cyst especially in areas where echinococcosis is endemic.

P1322 Incidental detection of microfilariae in a renal transplant patient

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Objectives: We intend to call attention to the incidence of tropical diseases in patients we attend in our European Transplant Units, and to the need of screening these patients before they received any kind of immunosuppression.
Methods: We used a protocol follow-up for detection of CMV antigenemia by imunocytochemical detection of an early structural protein (pp65) of the virus in peripheral blood leucocytes.
Results: By accident we detected high density of microfilariaemia, identified as Mansonella perstans, based in its morphologic characteristics and epidemiology.
Conclusions: No clinical signs or symptoms called attention to filariasis. Presumable due to the steroid therapy, eosinophilia was not present. In non immunocompromised patients, filariasis is often a benign situation, with no need for special therapy. Due to impaired immunity caused by triple immunosuppression, the patient was treated with mebendazole. We could not find any published report of filariasis in transplanted patients and we must be alert to tropical pathology in any patient, even asymptomatic, who came from endemic areas.

P1323 Hepatic capillariasis: an emerging zoonosis in Kolkata, India

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Objectives: Hepatic capillariasis caused by *C. hepatica* is an anthropozoonosis. Rodents are the main reservoirs of infection. The disease in man and animals manifests as hepatosplenomegaly and eosinophilia. The first authentic case of human capillariasis had been described by McArthur in 1924 from India following postmortem examination. Since then no case has been reported so far from India although cases have been reported from various parts of the world. Unlike other parasitic infections the disease cannot be diagnosed by conventional parasitologic stool examination. The only mode of diagnosis is liver biopsy which is not feasible always. The present study was undertaken on development of suitable serologic tests in animal models and its application in human beings suffering from hepatosplenomegaly and eosinophilia.
Methods: 102 black rats were examined macroscopically for the presence of hepatic and splenic lesions. Ova of *C. hepatica* had been collected and antigen had been prepared using standard technique. Co-agglutination (CoA) and passive hemagglutination (PHA) tests had been developed and standardized with the macroscopically positive and negative rodents. Twenty-four human sera had been collected from the patients suffering from hepatosplenomegaly and eosinophilia and CoA & PHA were applied to these sera.
Results: Ninety (88.2%) out of 102 black rats examined showed direct evidence of hepatic lesions (100%) and splenic lesions (16.6%). PHA appeared to be better than CoA for serodiagnosis when tested in rodents. Three (12.5%) out of 24 human samples tested showed antibody titre more than 1:160 and 1:256, respectively, following CoA and PHA test.
Conclusions: Tropical eosinophilia (TE) is very common in India particularly in the East and Southern parts of India and TE is one of the manifestations of hepatic capillariasis. Often patients with TE do not respond to antifilarial drugs also. This necessitated a serological study on hepatic capillariasis. Domestic black rodents is a very common association in both rural and urban India. This study highlighted that 12.5% human cases harbored high titre of antibody in their blood. Although this is a preliminary one, but this study indicates emergence of undetected cases of hepatic capillariasis in human beings if a suitable diagnostic method is developed.

P1324 Treatment of hydatid cyst with albendazole and praziquatel

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Objectives: The standard therapy of hydatid cyst is surgery but in nonoperable patients and multiple organ involvement medical therapy may be more useful efficacy of drugs especially in short duration in treatment of hydatid cyst is unknown. This study was carried out to evaluate the effect of combination therapy with albendazole and praziquantel in treatment of hydatid diseases.
Methods: In a nonrandomized clinical trial nine hydatid cyst patients with multiple organ involvement or postsurgical recurrence were treated with albendazole and praziquentel for 4 periods of 4 weeks duration with 2 weeks interval between them. The average follow up was 18 months response to treatment was assessed through the observation of the symptoms, radiologic (CT scan, sonography, conventional X-ray) and histologic study.
Results: Symptoms disappeared in 7 (77%) patients and improved partially in 2 (23%) patients. Radiologic assessment showed significant improvement in 5 (55%) and partial improvement in 4 (45%) patients. Histologic study was carried out on 2 patients and showed disappearance of the germinal layer.
Conclusion: Combination therapy with albendazole and praziquentel is effective in treatment of hydatid cyst and can be used as an alternative to surgery in disseminated and nonoperable cases.

P1325 The efficacy of niclosamide, mebendazole and praziquantel on 50 cases with *Taenia saginata* taeniasis and *Taenia solium* taeniasis

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Objectives: The recognition of the real efficacy of those drugs on treatment of taeniasis in our clinic.
Methods: We have treated during the period of 1982–2002, 50 patients, 29 males and 21 females of age 14–64-year-old with taeniasis (32 cases affected by *Taenia saginata* and 18 cases by *Taenia solium*) coming from 20 districts of our country. The diagnose was proved through coproparasitologic examination. As treatment we used niclosamide 25 cases (2 doses of 1 g within an interval of 2 h chewed thoroughly the drug before meal); praziquantel 12 cases (a single dose of 10 mg/kg) and mebendazole 13 cases (200 mg × 2/die for 3 days) at the end of treatment we have recommended 50–100 g magnesium sulfuric. Cases have been observed al least for 6 months having additional coproparasitologic examination 2–3 and 5–6 months after treatment as well.
Results: Niclosamide: the first cycle cured 16 out of 25 cases, 64%; the second cycle cured 4 out of 9 cases, 44.44% and the third cycle cured 1 out of 3 cases, 33.33% when the second cycle failed. The 2 cases when the second cycle failed and 2 cases when the third cycle failed with niclosamide, were cured using a cycle of praziquantel. Mebendazole: first cycle cured 7 out of 13 cases, 53.84%; second cycle cured 2 out of 5 cases, 40%. From 3 cases remained, 1 case was cured with a cycle of praziquantel and 2 cases with a cycle of niclosamide. Praziquantel: first cycle were cured 9 out of 12 cases, 75%; second cycle were cured 2 out of 3 cases when the first cycle failed, 66.66%. The remained case from the second cycle was cured through a niclosamide cycle.
Conclusions: None of the drugs has been acted 100% during the case treatment. Praziquantel and niclosamide resulted the first choice for treatment. Being twice failed with the same drug the substitution would be an efficient solution.

Brucella infections

P1326 Human *Brucella abortus* infection in Northern Ireland; the ongoing epidemic

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Objectives: A rise in cattle brucellosis has been mirrored by an epidemic of human brucellosis in Northern Ireland. Since 1998 50 cases of human brucellosis have been reported. Herein we describe the clinical features and outcomes of 20 of these patients (pts) who have been seen in the regional adult infectious diseases unit in Northern Ireland.
Methods: A retrospective review of 20 pts presenting with a history of acute brucellosis.
Results: There were 18 male pts and the mean age was 39.2 years. All pts acquired their infections through occupational exposure. Diagnosis occurred after screening at work in four pts. At diagnosis the commonest complaints were lethargy (95%), fever (85%), sweats (85%), and arthralgia (60%). Of these, four pts had focal disease including sacroiliitis (three pts), endocarditis (one pt), and orchitis (one pt). Of those with relevant investigations performed at diagnosis, 57, 29, 21 and O% had one or more abnormal liver function tests, a high C reactive protein, an ESR > 20 mm/h and an abnormal full blood count, respectively. Symptomatic pts received at least doxycycline 200 mg daily plus rifampicin 600–900 mg daily for 5–6 weeks. After initial appropriate treatment nine, six and five pts achieved complete cure, serological cure but ongoing symptoms, and relapse, respectively. Ten pts received more than one course of antibiotics. However, 50% of these had little evidence of ongoing infection.
Conclusion: Significant human morbidity with *Brucella abortus* infection has occurred concurrent with the rise in bovine brucellosis since 1998 and is ongoing. Many pts receive multiple antibiotic courses since sensitive diagnostic tests are unavailable. PCR tests are in development, which may differentiate between patients with active disease requiring additional antibiotics and those without.

P1327 A case of familiar brucellosis in Greece

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Objective: Brucellosis is a common disease in Greece, especially among persons with occupational animal relation. None the less, the appearance of the disease in many members of a family simultaneously, especially without occupational exposure, is rare. We describe an outbreak of brucellosis occurring in a family that had nothing to do with animals.
Patients: Four patients of a five-member family visited the Infectious Disease Hospital within a 10-days period with symptoms of brucellosis (fever, arthralgias, exhaustion, sweats). The father was 47 years old, the mother 41, the son 18 and the daughter 4 years old. All of them mentioned the consumption of unpasteurized goat cheese 2 months before. The second son of the family who remained healthy does not like cheese.
Results: Brucellosis was diagnosed by standard agglutination test, which was 1/1600 for the father, 1/400 for the mother, 1/320 for the daughter and 1/320 for the son. *Brucella melitensis* was isolated by blood cultures from the three members of the family; only the son had negative blood culture. The daughter had arthritis of the left elbow and hepatitis (SGPT = 833, SGOT = 479). The father and the son had slightly elevated transaminases (SGPT = 69 and SGOT = 44 the former, and SGPT = 71 and SGOT = 58 the latter). The mother had normal transaminases. The adult patients were treated with 1 g streptomycin for 21 days, 900 mg rifampicin and 200 mg doxocycline for 2 months. The child was treated with 20 mg/kg streptomycin for 15 days and 4 mg/kg doxocycline for 3 weeks.
Conclusion: The consumpion of unpasteurized dairy products is a common reason for brucellosis which can rarely cause a familiar outbreak.

P1328 Familial brucellosis: a report of four patients

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Objective: Brucellosis is a common infection in Turkey and we describe an outbreak of brucellosis occuring in a family in Konya in Turkey.
Results: Four cases of brucellosis in a 52-year-old-man, his 43-year-old wife, his 24-year-old and 16-year-old sons within 1 month. All of the cases admitted to the hospital with a history of pain and fever. All of the cases were diagnosed by brucella agglutination tests. Their test results were positive at 1/320, 1/320, 1/640 and 1/160 titers, respectively. *Brucella melitensis* was isolated from the blood cultures of the mother. Family brucellosis is attributed to cheese that they had eaten but the cultures from the cheese were negative and the family claimed that hey had kept the cheese in salt for more than 1 month. The mother and her 16 years-old-son were treated for 3 weeks with streptomycin and for 6 weeks with doxycycline and the boy recovered successfully but the mother relapsed after 45 days. The second therapy was made for 3 weeks with streptomycin, for 8 weeks with doxycycline and rifampin. The father and his 24-year-old son were treated for 6 weeks with doxycycline and ripampin combination. On the controls after 1 year all of them were healthy.
Conclusion: As brucellosis is very frequent in our area the animals must be vaccinated, milk and milk products must be under control and the people must be educated about this subject.

P1329 Brucella spondylitis

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Objective: To evaluate the clinical and laboratory findings, diagnostic evaluations and treatment of patients with brucella spondylitis.
Methods: Patients who had brucellosis followed between February-2001 to June-2002 were included to the study and evaluated for osteoarticular involvement prospectively.
Results: Eighty-six patients with brucellosis were included to the study. Mean age was 44.2 ± 17.7 (16–79) and 33 (38%) of the patients were female. Spondylitis was diagnosed in 26 (30%) of the patients. The mean age of

spondylitis cases were 57.3 ± 13 (24–74) and 11 (42%) of them were female. Patients with spondylitis were older than patients who did not have spondylitis ($P < 0.001$). The sexes affected equally. By the time from onset of symptoms, 12 (46%) patients were classified as acute and 13 (50%) were subacute brucellosis in the group with spondylitis; whereas 45 (75%) patients were acute and 14 (23%) patients were subacute in nonspondylitis group ($P = 0.016$) Spondylitis cases had higher ESRs compared with nonspondylitis cases ($P < 0.001$). *Brucella melitensis* were isolated from blood cultures from 10 (38%) of 26 patients with spondylitis. In MRI, two patients had cervical, one had thoracic, one had dorso-lumbar, 18 had lumbar, 3 had lumbosacral and one had sacral involvement. Additionally 14 (53.8%) patients had discitis and 6 (23%) patient had abscess. Four patients had epidural abscess and two patients had paravertebral abscess. In patients with cervical involvement, medullar compression was observed due to fragmentation in a patient and granuloma in the other patient. Both of these patients underwent surgical treatment. All patients received combined antibiotic therapy for 4–12 months.
Conclusion: Our study showed that; MRI evaluation has an important diagnostic value in brucellar spondylitis; surgical treatment may be needed in addition to medical treatment in cervical spondylitis as medullar and root compression is seen more often; the duration of antibiotic treatment has to be longer in spondylitis than systemic brucellosis without spondylitis.

P1330 Skeletal complications of brucellosis among children and adults in Babol, Iran 1998–2001

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Objectives: Skeletal complications are more common in brucellosis. Diagnosis of these complications may prevent surgical procedures. The aim of this study was to evaluate the skeletal complications of brucellsis in Babol, Iran.
Methods: This prospective study was conducted on patients with brucellosis in department of infectious diseases in Babol from 1998 to 2001. Skeletal complications and laboratory test results were noted. Data were analyzed by SPSS and proportions were compared with chi-square and Fisher's exact tests.
Results: 161 (37.6%) out of 431 subjects, had skeletal complications (94 male and 67 female). Mean age $\pm$ SD, for adults and children cases were 34 ± 17.6 and 9.5 ± 4.2 years, respectively. Among 127 adult cases, 79 (62%) had peripheral arthritis which was monoarthritis in 48 (37.8%) cases. Peripheral arthritis was seen in 32 (94%) of 34 cases of children, which was monoarthritis in 26 (76.5%) cases. Peripheral arthritis, and hip involvement were more common in children ($P < 0.05$), but spondylitis was more common in adult cases ($P < 0.05$). Diagnosis of brucellosis was performed in 14 (8.7%) cases after surgery. Normal ESR and positivity of Rheumatoid factor were seen in 80.7 and 15.5% cases, respectively.
Conclusions: Skeletal complications of brucellosis are similar with septic arthritis and rheumatologic disorders. Monoarthritis is the most common form of this complications. We recommend that in endemic regions, brucellois must be consider in differential diagnosis of all cases of monoarthritis.

P1331 Treatment of brucella spondylitis: a 3-year experience on the appropriate duration of therapy

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Objectives: To evaluate the efficacy of the treatment of vertebral osteomyelitis caused by *Brucella* spp.
Methods: We analyzed retrospectively 15 cases of brucella spondylitis which were diagnosed and treated in our Department from 1999 to 2002. Information on the age, sex and profession were included in the study. The diagnosis was based on the clinical signs and symptoms, the serological tests and the radiological findings, while in three cases *Brucella* spp. was isolated from the blood. In all cases an MRI of the vertebra was performed at the time of diagnosis and at least at the end of the treatment. In three cases, fine needle aspiration of the bone lesions was performed. The patients were given antimicrobial treatment with both rifampicin and doxycycline plus cotrimoxazole or ofloxacin.
Results: The total duration of treatment varies from 6 to 12 months. Cure was observed in 10 cases, relapse in one case while in four cases treatment is still on going. Cure was precised mainly clinically and secondary radiologically and serologically. In most cases, radiological findings were ameliorated by the end of treatment but did not disappear. No change in response rates was observed on patients given antibiotics for 6 months rather than 12 months. A long – term follow up is scheduled for these patients.
Conclusion: The optimal duration of brucella spondylitis treatment is discussed. Experience on diagnostic and therapeutic approach of a widely predominating, in the Mediterranean areas, disease involving the bones, is of value in order to define the appropriate length of therapy.

P1332 Brucella spondylodiscitis: serological diagnosis in patients with backache

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Objectives: Brucellosis is a pleomorphic zoonotic infection. Spondylodiscitis is one of frequent osteoarticular complications of Brucella infection in adults. The disease is a worldwide puplic health problem in many Mediterranean countries. The aim of this study is to present brucella infection as causative agent of spondylodiscitis.
Methods: We studied 72 patients with low backache in period 14 months (September-2001–November-2002). All patients were adults, satisfied the inclusion and exclusion criteria designed to exclude radiologically detectable congenital or degenerative causes of backache. The final diagnosis of Brucella was established by the history, radiographic findings and the detection of IgM and IgG to Brucella antibodies. Rose bengal test, Agglutination tube test and Immunoenzyme-assay were used.
Results: Six out of 72 patients (4males and 2females) were seropositive for brucellosis. All six patients had a history of animal contact or ingestion of raw milk or milk products. Mean age was 40 ± 12.2. The lumbar region was the commonly affected site. Neurological involvement in four patients was present, on admission. The most frequent constitutional complaints was arthralgia, fever and fatigue in all cases. Physical findings were diagnosed as follow: tenderness (4/6), hepatomegaly (2/6), splenomegaly (2/6) and arthritis (5/6). Abnormal laboratory findings were ESR > 50 mm/h, increasd CRP in all patients, though blood cultures were negative. Rose bengal test was positive in all patients. The tube agglutination test in higher titers than 1/640 was determined. The detection of specific IgM and IgG antibodies to brucella was the confirmatory tests for diagnosis of brucella infection. IgG in all patients were detected. IgM were positive in four patients. In two cases with negative IgM four-fold of IgG antibodies titers were found.
Conclusions: Low backache may be the only symptom in brucella of adults. Therefore bacteriological examination and especially the serological tests are necessary to establish the etiological diagnosis and determine the specific antimicrobial treatment.

P1333 Osteoarticular complications of Brucella infection. An 11-year retrospective

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Objectives: Brucella infection is endemic in Greece and in our state Lakonia in particular. We describe here the osteoarticular involvement of the infection in the hospitalized patients.
Methods: Between the year 1990 and 2000, 122 patients with brucella infection were admitted to our hospital. Ninety-three percent presented with acute infection diagnosed clinically and confirmed by the microbiology laboratory. Fifty-seven percent of the patients had localized infection.
Results: From the patients with localized infection 70% had osteoarticular involvement: 47% had low back pain, 8% sarcoilitis 28.5% asymmetric polyarthritis of large joints (hips, knees, sternoclavicular joins and shoulders) and 17.5 monoarthritis. The diagnosis was confirmed with serological test and cultures of synovial fluid. Blood cultures were positive up to 79% and the patients were treated with prolonged combined therapy with streptomycin – doxycyclin.
Conclusions: Osteoarticular involvement is by far the most common focal complication of brucella infection. The early suspicion together with the

proper diagnostic tests and prolonged treatment is crucial to the outcome of the infection in order to avoid relapses

P1334 Human mammary abscess caused by *Brucella melitensis*. A case report

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Objective: Soft tissue localized infections due to *Brucella* Spp. are uncommon in human. We describe a woman with a unilateral mammary abscess due to *Brucella melitensis*.
Method and Results: A 77-year-old woman was referred to hospital because of diffuse arthralgias, swelling and pain in her left breast. She was living in a rural area breeding animals. She was febrile with dry cough for 5 days, 3 weeks before admission, and was given a second-generation cephalosporin per os. In admission, she had a temperature of 37.5°C. The physical examination revealed hepatosplenomegaly with normal pulmonary findings. No lymph nodes were detected. There was a painful abscess at her left breast, which was initially thought to be an inflaming tumor, because of recurrent mastitis history. Blood tests revealed mild neutropenia (3250/mm^3 leukocytes, 40 polymorphonuclear, 45% lymphocytes), elevated transaminase levels, Wright agglutination test positive 1/320, ESR 70 mm/h. IgM and IgG antibodies for *Brucella* spp. were indicative of acute infection. Markers for breast and gastrointestinal tumors were normal. The breast ultrasound revealed thickening of the subcutaneous tissues and multiple abscess formations. An U/S guided FNA was performed, which yielded inflammatory cells, necrotic tissues but no cancerous cells. The biopsy material grew *Brucella* spp. and blood cultures were positive for *Brucella* spp. that were both identified as *Brucella melitensis*. The patient received doxycycline 100 mg twice a day per os for 8 weeks and streptomycin 1 g per day intramuscularly for 3 weeks. One week later the abscess had clinically disappeared and the control U/S revealed considerable decrease of the lesions. The patient received a 2-month treatment, and remained free of symptoms in clinical and laboratory follow up, for 8 months later.
Conclusions: Mastitis due to brucella is rarely reported in human. Usually the soft tissue locations are related to an injury, but in our case there was no history of trauma. The patient was probably exposed to the aspiration of infected aerosol. This could also explain the cough as a relevant symptom. The unusual location could be explained by the history of underlying pathology with recurrent episodes of acute mastitis. This case illustrate that since *Brucella* spp. is a slow growing organism in cultures the occupational history of people living in endemic areas, along with the standard tube agglutination tests may lead to early suspicion, diagnosis and appropriate choice of therapy.

P1335 An unusual cause of spontaneous bacterial peritonitis: *Brucella melitensis*

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Background: Spontaneous bacterial peritonitis is a serious complication in patients with chronic liver disease. The incidence of spontaneous peritonitis in cirrhotic patients is 15–20%. *Ecsherichia coli* and *Klebsiella pneumoniae* are the most frequently recovered pathogens. We present spontaneous peritonitis caused by an unusual pathogen, *Brucella melitensis*, in two patients with chronic hepatitis B and C, respectively. Abdominal distension, leg swelling and fever were the main complaints of the patients. Paracentesis was performed in both them. On examination of ascitic fluid, the findings were; leukocytes 400/mm^3 and 1000/mm^3 (%90 lymphocytes), protein 0.7 g/dL and 0.9 g/dL, LDH 46 U/L and 163 U/mL, respectively. No microorganism was detected in Gram's stain of the ascitic fluid. Brucella agglutination test was positive at a dilution of 1/2560 in both cases and it was also positive in ascitic fluid at a dilution of 1/32 and 1/320, respectively. In both cases *Brucella melitensis* were isolated from blood and ascitic fluid cultures. One of the patients treated with tetracycline and TMP-SXT, and the other, with streptomycin and TMP-SXT. With the treatment, both patients' condition improved.
Conclusion: In areas where brucellosis is endemic, in patients with lymphocytic ascitis brucellosis should be considered and appropriate microbiological and serological tests should be performed to confirm the diagnosis.

P1336 Neurobrucellosis: experience with 10 cases

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Objective: Brucellosis in Greece was and still remains a serious medical problem. Neurobrucellosis is a rare manifestation of the disease that, according to various studies, occurs in 2–10% of patients with brucellosis. Very few cases of neurobrucellosis have been reported in our country. The aim of this study is the presentation of the cases of neurobrucellosis in Infectious Diseases Hospital, where patients from all Northern Greece are attended.
Patients: We studied retrospectively 298 adult cases of brucellosis hospitalized in the adult department of the hospital during the years 1991–2002.
Results: Ten patients (3,3%) with neurobrucellosis were observed, eight men and two women, aged from 19 to 69 years. All men had occupational animal exposure and the two women mentioned consumption of unpasteurized dairy products. The patients had symptoms attributed to brucellosis for periods ranging from 15 days to 1 year. Three patients presented with symptoms of meningitis, five of meningoencephalitis and two of encephalomyelitis. All patients had CSF abnormalities. They had lymphocytic pleocytosis with 18–240 cells/mm^3, increased protein (75–571 mg/dL), reduced CSF/plasma glucose ratio (24–40%) and increased lactate (18–95 mg/dL). All patients had serum antibrucellar antibodies (standard agglutination and/or Coombs test positive in titers $\geq$1/60). Eight of 10 patients had CSF antibrucellar antibodies (standard agglutination and/or Coombs test positive in titers $\geq$1/40, and/or Elisa test positive). Brucella was isolated from blood in three of 10 patients and from CSF in two of 10 patients, respectively. All patients were treated with a combination of three antibrucellar drugs (streptomycin, doxocycline and rifampicin or cotrimoxazole) for two to 6 months. The outcome was perfect in all patients with meningitis and meningoencephalitis, but in the two patients with myelitis there was residual motor disability.
Conclusion: Neurobrucellosis is a rare but serious complication of brucellosis and a diagnostic delay can enhance residual disturbances.

P1337 Forty-six Brucellosis cases in which 70% of blood cultures are positive

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Objective: Forty-six patients are followed up with the diagnosis brucellosis at Haseki Education and Research Hospital.
Methods: The patients were diagnosed according to serological and cultural methods and also their clinical symptoms. In 70% of the patients the blood culture (BACTEC 9050 Becton Dickinson) were positive. Twenty-nine of the patients were followed up as inpatients. The most frequent symptom fever was seen in 31 cases. The other frequent symptoms were sweating and backache. One quarter of the patients have the history of ingestion of unpasteurized diary products, which is an important route of transmission. Sixty percent of the patients had these complaints lasting over 1 month before they were diagnosed. In nine cases leucopenia, seven cases spondilodiscitis, four cases pancytopoenia were seen. In one case, *Brucella* spp. was isolated from the cerebrospinal fluid and blood. The most of the cases were treated by doxycycline and rifampin. No relapses were seen in the treated cases.
Result: The diagnosis of brucellosis is difficult since it is a long-lasting and silent disease and it has important complications. It should be remembered that brucellosis is one of the most frequent causes of fever of unknown origin in our country. The mean growth period of *Brucella* spp. in automatized blood culture systems, was found to be 3.3 days.
Conclusion: The length of the time interval between the symptoms and the diagnosis shows brucellosis is hard to be diagnosed. Automatized blood culture systems are very useful which provide a high ratio of growth for *Brucella* spp.

P1338 Comparison of diagnostic value of Standard Tube Agglutination Test with the ELISA Ig G and Ig M in patients with brucellosis

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Objectives: Brucellosis is one of the major zoonotic diseases in many parts of the world, especially in Mediterranean and Middle East countries. It is also endemic in Turkey. Since it affects many organs and the symptoms are nonspecific, the diagnosis by clinic findings is difficult and may be easily missed. However, positive blood cultures occur in 10–70% of suspected infections, serological tests play a major role in diagnosis when the disease can not be detected by blood culture. Many serological tests have been used for the diagnosis of human brucellosis. The most commonly used tests are the standard tube brucella agglutination test (SAT), the Rose Bengal test, complement fixation test and ELISA. The aim of this study was to compare the diagnostic value of the brucella SAT, with ELISA (brucella specific IgG and IgM) tests in patients with brucella bacteremia.
Methods: The study was done on 32 patients with brucellosis who had positive blood and/or bone-marrow cultures for *Brucella* species, and 20 healthy individuals as control. Fifty-two serum samples from both groups were tested for brucella specific IgG and IgM antibodies by ELISA using commercial kit (Novum, Germany). Same samples were also tested by SAT
Results: In the patients group, 30 of 32 samples gave positive result by SAT (titer >1/160). Of the same group, IgG and IgM antibodies by ELISA were positive in 26 and 32 patients, respectively. In 24 of the patients both IgG and IgM antibodies were detected. From 20 control sera, all were negative (titer <1/80) in SAT, 1 in ELISA IgG and 3 in ELISA IgM were positive. Positive predictive value of SAT was calculated as 100.0% and the negative value was as 90.9%. Positive and negative predictive values for ELISA IgG were 96.3 and 76.0%, and for ELISA IgM were 90.9 and 89.5%, respectively. Sensitivity and specificity rates of SAT, ELISA IgG and ELISA IgM tests were found as 93.7, 100.0, 81.3, 95.0, 93.8 and 85.0%, respectively.
Conclusion: The overall data showed that the sensitivity of SAT and ELISA IgM tests were nearly equal, but the sensitivity of ELISA IgG was lower than the other two. On the other hand the specificity of SAT was higher than that of both ELISA IgG and IgM. According to the results of this study, SAT can be preferred to ELISA because it is cheap and easy applicable.

P1339 Evaluation of growth time in *Brucella* spp.

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Objectives: The isolation of *Brucella* species from blood may be achieved by using classic culture techniques but detection of the organism is difficult due to its slow growth. The detection time of brucella can take up to 30 days by using Castaneda blood culture method. Automated blood culture systems have reduced the growth time of brucella. In this report, we would like to contribute our experience on detection time in the isolation of *Brucella* species from blood, by use of Bact/ALERT and BACTEC 9240 blood culture systems.
Methods and results: Thirty *Brucella* spp. were isolated from 35039 blood culture sets between 1995 and 2002. Blood cultures were recovered between 1.8 and 3.7 days in BacT-Alert blood culture system, while 2.1–3.8 days in BACTEC 9240 system.
Conclusion: *Brucella* spp. can be recovered minimum at 7 days in traditional procedures while the maximum time is 3.8 day for automated blood culture system. We concluded that automated blood culture systems could isolate *Brucella* spp. in rapid and efficient way.

P1340 The role of adenosine deaminase activity and soluble interleukin 2 receptor levels in the discrimination of acute and chronic brucellosis

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Objectives: We aimed to investigate the role of adenosin deaminase (ADA) activity and soluble interleukin-2 receptor (sIL-2R) levels in the differentiation of acute from chronic brucellosis.
Methods: ADA levels were measured in the sera of 17 acute brucellosis, 12 chronic and 11 healthy volunteers. sIL2R levels were measured in the sera of 15 acute brucellosis, 12 chronic and 10 healthy volunteers. ADA activity was assayed according to the method of Giusti. Measurement of sIL-2R was made using immuno enzymometric kit by Immunotech International.
Results: In acute brucellosis group (AB), ADA activity and sIL-2R level were 71.24 ± 29.48 and 126.20 ± 92.43, respectively. ADA activity was found as 51.16 ± 35.85 and sIL-2R level was 144.38 ± 103.28 in chronic brucellosis group (CB). In healthy controls ADA activity was 12.46 ± 2.06 and sIL-2R level was 116.88 ± 24.16. ADA activity was found higher in both groups than controls ($P < 0001$). However, There was no statistically difference in differential diagnosis between acute and chronic brucellosis ($P > 0.05$). sIL2R levels in sera were not significantly different among acute, chronic brucellosis and controls ($P > 0.05$).
Conclusion: This study showed that both ADA activity and sIL-2R level are not informative in the discrimination of acute and chronic brucellosis.

P1341 The role of adenosine deaminase activity and soluble interleukin 2 receptor levels in cerebrospinal fluid in differentiation tuberculous meningitis from bacterial and viral meningitis

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Objectives: Tuberculous meningitis is difficult to be distinguished from onset of viral and bacterial meningitis with low cell-count in cerebrospinal fluid. In this study, it was investigated the role of adenosin deaminase (ADA) activity and soluble interleukin-2 receptor (sIL-2R) levels in the differentiation meningitis especially of tuberculous from bacterial and viral meningitis.
Methods: ADA and sIL-2R levels were measured in the CSF samples of 16 tuberculous meningitis, 37 bacterial meningitis, 18 viral meningitis and, and 35 CSF samples without any inflammation as controls. ADA activity was assayed according to the method of Giusti. Measurement of sIL-2R was made using immuno enzymometric kit by Immunotech International.
Results: In tuberculous meningitis group (TBM), cerebrospinal fluid ADA activity and sIL-2R level were 29.00 ± 7.11 U/L and 1509.10 ± 1029.12 U/mL, respectively. ADA activity was found as 14.02 ± 10.91 U/L and sIL-2R level was 273.68 ± 366.94 U/mL in acute bacterial meningitis group (BM). In viral meningitis group (VM), CSF ADA activity was 2.84 ± 1.77 U/L and sIL-2R level was 40.03 ± 36.18 U/mL. In control group, ADA activity was found to be 1.44 ± 0.78 U/L and sIL-2R level was 9.56 ± 1.68 U/mL. The mean CSF ADA activity and sIL-2R level were significantly elevated in TBM group as compared with BM and VM and control groups ($P < 0.001$). In differentiation TB from other meningitis, by using 9 U/L as a cut-off value of ADA activity we showed that the test had 100% sensitivity, 75.6% specificity and 42.1% positive predictive value (PPV). When cut-off value was taken as above 500 U/mL for sIL-2R, we determined the sensitivity, specificity and PPV as 91.7, 96.3 and 78.6%, respectively.
Conclusion: This study is showed that both ADA activity and sIL-2R level are useful in the differential diagnosis of TBM from BM and VM.

Organism carriage, prevalence, and incidence

P1342 MRSA awareness and nasal carrier rate among newly recruited staff nurses in a new tertiary care specialist hospital at Taif, Saudi Arabia

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Objectives: To evaluate the awareness of MRSA among newly recruited staff nurses. To know the nasal carrier status of *S. aureus* and MRSA among them. To record the base line antibiotic susceptibility pattern of *S. aureus* among the isolates from the carriers.
Methods: This study was designed as a part of education on infection control to newly recruited staff nurses (216) in King Abdul Aziz Specialist Hospital, Taif. A written questionnaire was responded by staff nurses relating to the problem, source, method of spread, suspect of an outbreak and measures to be taken to prevent and control MRSA in a newly commissioned hospital. Nasal swab was taken from each staff nurse and inoculated on to mannitol salt agar and blood agar. *S. aureus* was identified by conventional and latex agglutination methods. Oxacillin (1 μg disc) resistance was taken as less than 10 mm screening and beta-lactamase test was done by disc diffusion method and cephalosporin disc method, respectively. Staff nurses were educated in terms of source, spread and suspicion of an outbreak and preventive measures to be taken in such eventuality.
Results: Respondents had difficulty in suspecting an outbreak. Of 216 nasal swabs, 43 (19.9%) showed *S. aureus*. All 43 isolates were susceptible to oxacillin (MSSA), vancomycin, amoxy-clavulanic acid, gentamicin, cotrimoxazole and rifampicin. Thirty-eight (88.3%) isolates were penicillin resistant and was due to beta-lactamase production. Two isolates showed inducible erythromycin resistance and one constitutive resistance.
Conclusions: *S. aureus* nasal colonization among staff nurses was 19.9%. None revealed MRSA colonization. Education in suspecting outbreak needs to be highlighted.

P1343 Prevalence of MRSA in European hospital populations: implications for empiric therapy

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Objectives: The prevalence of *Staphylococcus aureus* (SA) and methicillin-resistant SA (MRSA) can vary between regions and patient groups. If the incidence of MRSA is high, nonanti-MRSA empiric therapies could have serious consequences for infected patients.
Methods: Data derived from the TSN Database, an electronic surveillance system that collects routine susceptibility test results from hospital laboratories in France (Fr; 63 sites), Germany (Gy; 169 sites), Italy (It; 48 sites), and Spain (Sp; 21 sites) were used in this analysis. Data from 1 January 2000–31 August 2002 were used to define the overall incidence of SA and MRSA by patient location (inpatient, ICU, surgical), age group (<18, 18–64, >64 years), and site of infection (blood or lower respiratory tract [LRTI]), as a proportion of all nonrepeat bacteria isolated and susceptibility tested. Contemporary NCCLS breakpoints were used for all countries except Fr, in which CA-SFM breakpoints were used.
Results: The incidence of SA varied little by age and location comprising 7.5–21.5% of all isolates. The incidence of SA was higher in LRTI comprising 19.1–24.8% of isolates in Fr and 19.2–29.1% of isolates in It, depending on patient location. The overall incidence of MRSA in each country was Fr (40.2%), Gy (16.5%), Italy (48.2%), and Spain (38.5%). For all patient locations in each country, the incidence of MRSA increased with age. The highest levels reported were in patients >64 years in It (52.9–67.6% of SA), Fr (39.1–48.0% of SA), and Sp (24.4–44.7% of SA). By patient location, 39.9–57.5% of SA from LRTI in Sp and It were MRSA, compared with 35.5–42.8% in Fr and 8.0–16.4% in Gr. The highest levels of MRSA as a proportion of all bacteria isolated were recorded in It, comprising 10.8, 15.4, and 14.7% in inpatient, ICU, and surgical patients, respectively.
Conclusions: The variation in MRSA rates suggests that empiric antimicrobial therapy must be tailored to account for patient age, hospital location, and site of infection. In particular, in patients >64 years and for LRTI, the high prevalence of MRSA suggest that covering MRSA when choosing an empiric therapy could be prudent. Studies are required to test whether such antibiotic policies would positively impact patient outcomes and costs.

P1344 The first molecular survey on mycoplasma infections in inflammatory vs. noninflammatory arthritis in Iran

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Background: Mycoplasma have been debated to be associated with acute and chronic arthritic diseases.
Aim: We devised a survey based on a statistical sample to determine the significance of mycoplasma in inflammatory vs. noninflammatory arthritis in Iranian patients.
Methods: Synovial fluid samples were collected from knees of 99 patients with arthritis, all of which fulfilled the standard criteria for diagnosis. Patients were categorized as inflammatory (59) and noninflammatory (40) subjects. An aliquot of each synovial fluid was placed in lysing buffer, followed by DNA extraction. An optimized PCR protocol was then performed to detect a 280-bp 16S rDNA specific for *Mycoplasma* genus. After confirmatory restriction digestion of PCR product, the densitometric data of visualized bands were analyzed statistically and the *P*-values lower than 0.05 considered significant.
Results: From the 99 patients under study, mycoplasma DNA was detected in 5 (8.5%) of 59 samples from inflammatory arthritis patients, and in 6 (15%) of 40 samples from noninflammatory arthritis patients. Epidemiological parameters including sex, age, previous antibiotic therapy and smoking did not showed significant correlation with PCR results.
Conclusions: This study advocates the association of mycoplasma infections with arthritis, either inflammatory or noninflammatory. However, more studies are required on determining comparative frequencies of mycoplasma vs. other possible bacteria.

P1345 Evaluation of anti-*Chlamidia antibodies in* patients with coronary artery disease

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Objectives: Atherosclerosis (AS) is important laying cause of coronary artery disease (CAD). Common risk factors only contain almost half of patients with AS. A potential relationship between infectious factors and AS has been suggested recently, and some seroepidemiological evidences have suggested the role of *Chlamidia* pneumonia (CP) bacteria in the etiology of AS.
Methods: This study has performed as retrospective case-control on the blood sample from 56 cases that they were referred to Ali Ebn Abitaleb hospital, that group (1) including 29 cases with acute myocardial infarction, and group (2) 29 cases with chronic stable angina. Control group includes 29 healthy matched individuals (group 3).The case and control groups matched from sex, age and major coronary risk factors. After assembling of samples, IgG antibody concentration against CP measured in sera by ELISA. Serum antibody ranges higher than or equal of 5 unit/mL carried as positive and lower ranges as negative. Data analyzed by INSTAT soft ware and parametrical Kruscal–Wallis test, χ^2-test and Dunns multiple comparison test. Statistical significant level was $P < 0.05$.
Results: In the group (1) positivity of anti CP antibody concentration became 100%, group (2) 65.6% and in control group 31%. The difference between these results became significant with $P < 0.0001$. Also median concentration of antibody in these groups accounted as 34.7, 11.4 and 3.6 μM/mL, respectively, that difference between these medians were significant with $P < 0.0001$.

Conclusions: These results show that there is anti CP antibody in considerable percent of patients with CAD especially in patients with acute myocardial infarction. Therefore it can be supposed that there is a relationship between CP and CAD or its complications, and expected that can decrease in incidence of out come of CAD by antibiotic drug therapy against CP.

P1346 Positive association between essential hypertension and *Chlamydia pneumoniae* but not Epstein–Barr antibodies

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Objectives: Conflicting data exist regarding the association between essential hypertension and *C. pneumoniae* seropositivity. In our study, *C. pneumoniae* IgA and IgG antibody titers were measured in 306 hypertensive patients and 45 normotensives (control group). Epstein–Barr virus antibodies were also estimated in order to clarify whether a possible relationship between hypertension and *C. pneumoniae* is specific or not.
Methods: *C. pneumoniae* IgA and IgG antibody titers were measured by microimmunofluorescence method (MIF). Elementary bodies of *C. pneumoniae* TW 183 strain, were used as antigen. IgA ≥ 40 and IgG ≥ 80 were defined as elevated titers. Epstein–Barr antibodies (VCA-IgG, VCA-IgM, EA, EBNA) were measured by the ELISA technique. All subjects underwent casual blood pressure readings by sphygmomanometer and 24 h ambulatory blood pressure monitoring. The participants by selection were free of any cardiovascular disease evidence. Smoking status, age, and gender were recorded in each patient. Serum cholesterol, triglyceride, and glucose were determined.
Results: There was no statistically significant difference between the two groups in age, smoking status, cholesterol, triglyceride and glucose serum levels. A total of 172 hypertensives (56.2%) and 6 normotensives (13.3%) had IgA titers ≥ 40 (crosstabs $P < 0.005$). A total of 218 hypertensives (71.2%) and 20 normotensives (44.4%) had IgG titers ≥ 80 (crosstabs $P < 0.001$). No difference in Epstein–Barr antibody levels was found between hypertensives and normotensives.
Conclusions: A positive association between *C. pneumoniae* seropositivity and essential hypertension was found suggesting high frequency of chronic *C. pneumoniae* infection in hypertensives.

P1347 Association between *Chlamydia pneumoniae* antibodies and Crp levels in hypertensive patients

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Objectives: Controversy exists concerning the involvement of *C. pneumoniae* in atherosclerosis via a chronic inflammatory process. The aim of the present study was to investigate the association between C-reactive protein levels as an inflammatory marker and *C. pneumoniae* antibody titers in a selected population of hypertensive patients.
Methods: *C. pneumoniae* IgG antibodies were evaluated in 348 hypertensives by the microimmunofluoresence method (MIF). Elementary bodies of *C. pneumoniae* TW 183 strain, were used as antigen. IgG ≥ 80 were defined as elevated titers. All subjects underwent casual blood pressure readings by sphygmomanometer and 24 h ambulatory blood pressure monitoring. Smoking status and age were recorded in each patient. Hypertensives having clinical or laboratory evidence of previous or present infection and cardiovascular disease were excluded from the study.
Results: *C. pneumoniae* seropositive and -negative patients did not differ in age and smoking status. In IgG seropositive hypertensives (IgG ≥ 80) the average of CRP was 0.68 ± 1.14 mg/dL whilst in IgG seronegatives was 0.48 ± 0.34 mg/dL ($P < 0.02$).
Conclusions: A positive association was found between elevated *C. pneumoniae* IgG titers and CRP levels in hypertensives. This association adds to the body of evidence that the possible underlying pathophysiological mechanism of *C. pneumoniae* microorganism is inflammation.

P1348 Identification and biotyping of *Brucella* spp. isolated from humans in central Anatolia

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Objectives: Brucellosis is a very widespread zoonotic diseases in some regions of Turkey. Prevention of brucellosis in man is dependent on the control and eradication of the disease in animals. In this study, we identified and biotypes of *Brucella* spp. isolated from the patients admitted to our department from various regions of central Anatolia, we aimed to determine clinical variations according to the biotypes and to get some epidemiological data of our region.
Methods: *Brucella* spp. were isolated from either blood, bone marrow or cerebrospinal fluid cultures of patients by using BACTEC 9050 system. Isolates were evaluated on the basis of requirement of CO2 for growth, production of H_2S, sensitivity to thionin and basic fuchsin dies. Tbilisi phage typing was also used to determine the species. Biotyping was performed by agglutination method by using A–M monospecific antiserums. Identification and biotyping of *Brucella* species were performed at *Brucella* Laboratory of Pendik Veterinary Control and Research Institute.
Results: A total number of 70 *Brucella* spp. isolated from different patients were included in the study. All *Brucella* isolates were identified as *B. melitensis*. It was found that 65 of the isolates (92.8%) were *B. melitensis* biovar 3, and 5 isolates (7.1%) were *B. melitentis* biovar 1. Because of the number of other biovars accept than biovar 3 were too low, we could not compare the clinical variation.
Discussion: *B. melitensis* biovar 3 was the most frequently found biotype in our region. Biotyping of *Brucella* species will give us important data for national control and eradication programs of brucellosis In order to evaluate the clinical importance of different biotypes of *Brucella* more studies will be needed.

P1349 Detection of anti-*Brucella* antibodies in the sera of slaughterhouse and veterinary farm staff by the Wright and ELISA methods

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Introduction: Brucellosis is an important public health problem that occurs worldwide. It causes economic losses among domesticated animals used as a sources of meat and dairy products and is frequently transmitted from animals to man in areas where the disease is enzootic. Direct contact with contaminated tissues of cattle presumably responsible for a majority of cases among livestock producers, livestock market employees and veterinarians. Brucellae are facultative intracellular bacilli. For seroepidemiological studies the standard tube agglutination test widely used. The main goal of this study is seroepidemiological studies of anti-*Brucella* antibodies in the sera of butchers and veterinary farm employees.
Material and methods: Questioners were prepared and 305 blood samples were obtained from employees by random. Anti-*Brucella* antibodies were measured from the sera by the standard agglutination test (Wright test). For determination of the class of antibodies and for identification of false negative results ELISA, 2-ME Wright and Coombs Wright tests were carried out.
Results: In 305 samples, 36 (12%) samples the Wright agglutination test were positive and the titer was $>1/160$ and in 43 (14%) samples the titer was 1/80, in the 15 (5%) samples the titer was 1/40 and at the rest of cases no agglutination was observed. The ELISA and 2-ME results were indicated that anti-*Brucella* IgM antibodies were found in 12 (4%) and anti-*Brucella* IgG antibodies were found in 24 (8%) of positive samples (from 36 samples noted above).
Discussion: The results were indicated 36 (12%) employee due to their occupation potentially exposed with contaminated materials. The positive results were indicated at least 12 (4%) persons suffer from acute brucellosis and 24 (12%) persons suffer from subacute or chronic brucellosis. It was recommended for prevention of disease adherence to biosafety and precautions should be noticed and in high risk personnel vaccine should be designed and used. More details will be discussed in conference.

P1350 Epidemiology of *Campylobacter jejuni/coli* infections in the Zenica-Doboj Canton, Bosnia and Herzegovina

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Objective: It has previously reported by this author about some interesting epidemiological features of *Campylobacter jejuni/coli* infections after the war in this region. This prompted us to carry out a survey to determine in its prevalence, distribution by demographic features and antimicrobial resistance in the 1999–2001 period.
Methods: It was examined 5426 consecutive human stool samples for the presence of *Campylobacter* spp. during 1999–2001. The strains were identified using the standard microbiological methods and were tested by disc diffusion method to eight antimicrobials according to NCCLS.
Results: A total number of 40 (75.5%) *C. jejuni* and 13 (24.5%) *C. coli* nonrepeated isolates were analyzed. More than half of isolates, 30 (56.6%) were from urban dwellers, resulting in a rate of 6.7 cases/100 000/year in urban population compared with 4.2 in rural one. *Campylobacter* isolates mainly obtained from children under 6 years of age, 42 (79.2%), resulting in far off highest rate of 41.4/100 000/year in this age group. M/F ratio was 1.1 : 1, but gender-specific incidence was higher in male, 8.3/100 000/year vs. 3.8. Infections mostly appeared in autumn, 71.7% (38 isolates). The frequencies of resistance for all tested antibiotics were doubled in 2001, comparing 1999. The resistance rate to ciprofloxacin in children under 6 years of age was 27.3%. There was high erythromycin-resistance (>30%) in all subset analyzed and higher in *C. coli* isolates (46.2% vs. 26.3%).
Conclusions: Although *Campylobacter* isolation rate was low, as in developed countries, the high proportion of infections in children under 6 years of age in urban zone is an epidemiological features similar to that of developing countries. It might be a consequence of recent war in this region. The frequency of resistance to ciprofloxacin in the youngest for which quinolone use is not recommended was surpassingly high. Also, there was persisted high erythromycin- resistance, although this drug participates with only 2.2% in antibiotic usage in this region. Thus, overuse of theses drugs in humans probably did not the reason for such high frequencies of resistance. Campylobacteriosis in this region is a public health concern, but not in the terms of the numbers reported cases, but of distinctive epidemiological features. Thus, research is required to identify the reservoir of *Campylobacter* spp. in the environment.

P1351 Prevalence of certain infectious diseases among economical immigrants in Greece

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Objective: To estimate the seroprevalence of certain infectious diseases in a large cohort of economical immigrants that visited our hospital.
Methods: During a 5-year period (1998–2002), 3.215 consecutive immigrants visited our hospital and they were submitted to laboratory testing for certain infectious diseases, according to provisions in Greek legislation. For purpose of analysis, we divided the studied population into three groups, according to their geographical origin: 771 (24%) of them were Albanians, 675 (21%) came from Asia or Africa and 1769 (55%) came from East European Countries. Beyond the tests for certain infectious diseases, required by Greek legislation, for issuance of a health certificate (Hbs Ag, stool examination for parasites, thorax radiography), we did a further anonymous testing of the serum samples for hepatitis markers, as well as for antibodies against HIV I/II and HTLV I/II. For all the serological testing, we used a commercial enzyme immunoassay (EIA, Roche Diagnostics, Mannheim, Germany). All the positive samples were examined twice.
Results: No one of the serums tested was found to be positive for antibodies against HTLV I/II, whereas two of the serums tested, coming from the group of Asian-Africans were positive for antibodies against HIV I/II, something that we confirmed by the Western Blot method. The testing for HCV markers showed that 5.4% of the East Europeans, 2.6% of the Albanians and 2.9% of the Asian-Africans were positive for antibodies against HCV. We also found that 17.6% of the Albanians, 4.9% of the Asian- Africans and 5.3% of the East Europeans were Hbs Ag(+). The prevalence of HbsAg as well as of HCV infection for the Greek population is about 2.5–3%. The results of our study are similar to those of other studies that took place in hospitals of other areas in Greece.
Conclusions: The prevalence of HCV and HBV infection in the population of economical immigrants in our country is higher than that of the Greek population. We also notify the high prevalence of HbsAg among the Albanians.

P1352 Occupational exposure of health care workers to blood and body fluids

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Objective: To determine the frequency and the epidemiological characteristics of occupational exposure incidents among healthcare workers (HCWs).
Methods: A survey was conducted in Ataturk University Medical School Hospital using a questionnaire form. Each questionnaire was completed during an interview with the HCW. For statistical analysis, categorical variables were compared using the chi-square or Fisher's Exact Test.
Results: A total of 386 HCWs (217 medical doctors, 124 nurses, 45 laboratory technicians) were included in the study. Mean ages and working periods were as follows for doctors, nurses and laboratory technicians, respectively: 38.2 ± 11.0 and 9.3 ± 6.5 years, 35.5 ± 12 and 8.9 ± 5.8 years, 38.0 ± 8.0 and 9.9 ± 7.3 years. A total of 313 (81.1%) of HCWs reported one or more exposure incidents (78.3% of doctors, 91.1% of nurses, and 66.7% of laboratory technicians, $P = 0.000$). Blood and blood products were involved in 84.5% of exposure incidents; body fluids, excrement and other substances accounted for 3.9, 2.4, 9.2% of cases, respectively. The most common route of exposure was sharp and needle-stick injury, 46.6% of all HCWs, followed by contact to open skin break (24.4%) and splash to mucous membranes (19.2%). The first two was apparently more frequent among the nurses, $P = 0.000$ and $P = 0.001$, respectively. Of the 313 exposed HCWs, 12.8% had one exposure history, while 17.6% had two, 15.0% had three and 54.6% had four or more. Only 58.2% of the HCWs were vaccinated against hepatitis B virus (HBV), 17.9% were unaware of their own serological status to HBV, 17.4% reported that they were immune because of past HBV infection, 1.6% were carriers and 4.9% were still seronegative. For HCV and HIV, 37.6% were unaware of their own serological status, 62.2% were seronegative, and 0.3% had chronic HCV infection. No HCWs serpositive for HIV or exposure to HIV positive blood and body fluids were recorded. Of the exposed HCWs, 3.8% had been infected with HBV and 0.03% with HCV, related to the exposure. The doctors on surgical wards were more exposed (88.5%) than the doctors on medical wards (69.0%), $P = 0.001$. However, the rates of doctors seropositive for HBV were similar (15.0% vs. 18.3%, $P > 0.05$) in these wards.
Conclusion: This results indicate the need to educate of HCWs about the risk of occupational exposures, preventive strategies and accurate post exposure follow up, in addition to pre-employment screening for blood-borne pathogens and vaccination against HBV.

P1353 HIV infection among women who immigrated to Italy from outside the European Union (EU): epidemiological, clinical, and therapeutic features, compared with a population of male immigrants

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Objective: To assess the frequency and epidemiological, clinical and therapeutic features of HIV infection in women immigrated from outside the EU vs. a comparable population of male immigrants followed during years 2001–2002.
Methods: Among the 1027 HIV-infected patients (p) followed during the last 2 years, 74 (7.2%) come from outside the EU. Adherence to prescribed medication was estimated by questionnaires, and a monthly direct drug accountability.
Results: Compared with all remaining HIV-infected p of our cohort, immigrants include a greater percentage of women, an increased frequency of heterosexual and perinatal transmission, and a shorter period of known seropositivity. Opposed to male p, female immigrants were more frequent (39 vs. 35 p), significantly younger (mean age 30 vs. 34 years; $P < 0.001$), more frequently exposed to sexual transmission vs. drug addiction ($P < 0.04$), but they detected HIV infection more frequently after migration, since HIV was acquired and/or discovered in our country ($P < 0.04$). The shorter history of HIV disease led to a lower incidence of AIDS (8 vs. 15 p; $P < 0.04$). Compared with male immigrants, an effective access to anti-HIV therapy, characterized by an earlier initiation, a better compliance, and a lower

frequency of discontinuation due to untoward events ($P < 0.05$, $P < 0.01$, and $P < 0.05$, respectively), leads to the present higher mean CD^{4+} count (373 vs. 311; $P < 0.03$) and lower mean viremia (3.4 ± 0.4 vs. 3.9 ± 0.8 Log_{10}; $P < 0.001$) among women, besides a more frequent suppression of HIV replication (71.8% among female vs. 54.3% of males).
Discussion: Migration to Italy is a recent occurrence and involves p coming from areas endemic for HIV infection or p exposed to HIV after their arrival in the EU because of their economic, social and sanitary situation. A timely management of HIV disease is an emerging issue among recently immigrated p, due to epidemiological reasons (extra-EU p are > 7% of HIV-infected p at our center), and the socioeconomic and psycho-cultural features which accompany the migration project and the detection of an infectious, chronic and life-threatening disease, needing life-long therapy and controls. Although immigrants are expected to show a clinical-immunologic course of HIV disease matching that of Italian p, prevention projects need to stress the need of timely screening and therapy of HIV infection, and an adequate counseling especially when female p are involved because of their predominant sexual exposure and childbearing potential.

P1354 The screening of throat, conjunctiva and faces carrier stage among nursery children

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Objective: 52 children who are attending at a nursery school serving only for the workers of the Haseki Education and Research Hospital are examined for their throat, conjunctiva and faces carrier stage.
Methods: For throat cultures, blood agar and chocolate agar media were used. Whereas for conjunctiva cultures, chocolate agar medium and thioglycollate broth were used. From the throat cultures we identified 23 coagulase-negative *Staphylococcus*, 21 *S. aureus*, 12 beta-hemolytic *Streptococcus*, 10 *Haemophilus haemolyticus*, 5 *Haemophilus influenzae*. From the conjunctiva cultures, we identified coagulase-negative staphylococci and *S. aureus*. The stool specimens were inoculated Endo, *Salmonella–Shigella* agar media and Selenite F broth in order to screen *Salmonella* and *Shigella* spp. This study covered 22 children who were aged between 1 and 3 years, whereas 28 were older than 3 years.
Result: In only one case, ampicillin/sulbactam resistant *Salmonella* spp. was detected. This case was 2.5 years old, attending at the nursery school for 1 year. *N. meningitidis* was identified from the throat culture of one case. This case was 5-year-old and attending at the nursery school for 1 year. With this study we have screened the carrier stages of the children and necessary treatment were given.
Conclusion: In public places, some bacteria can easily cause severe outbreaks, therefore screenings sometimes should be enlarged including all the family members.

P1355 Nasopharyngeal carriage of *Streptococcus pneumoniae* in infants and young children in three different settings in Warsaw

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Objectives: The aim of the study was to analyze the risk factors of nasopharyngeal carriage of *S. pneumoniae* in selected settings of children under the age of 5. It was also to determine the most prevalent serotypes among pneumococci isolated from asymptomatic children and to characterize the penicillin-resistant isolates (PRP).
Methods: A total of 223 children were examined between November 2000 and April 2002 in selected settings in Warsaw: day care center (DCC, $n = 58$), orphanage (OR, $n = 107$), and the family children (FC, $n = 58$). Clinical data, including information on the antibiotic treatment within the 3-month period before the beginning of the study, were collected with the use of a specific questionnaire. Nasopharyngeal swabs were processed and *S. pneumoniae* was identified by conventional microbiological methods. The MICs of antimicrobials were determined by broth microdilution method according to the NCCLS guidelines. Serotypes of the isolates were determined by the Quellung reaction. PRP isolates were typed by pulsed-field gel electrophoresis (PFGE).
Results: Altogether 114 *S. pneumoniae* isolates were identified; 36 from DCC, 65 from OR, and 13 from FC. Thus, the carrier rate of the organism was 62, 61, and 22%, respectively. The most frequent serotype in DCC was 6B (49%), followed by 19F (31%). The 6B serotype was also highly prevalent in OR (52%), however, in this setting it was followed by 23F (31%). Isolates from FC belonged to various serotypes, represented mostly by 1–2 isolates each. Nine of the isolates were resistant to penicillin (one isolate in DCC, eight strains in OR), and all these belonged to serotype 23F. All the children colonized by PRP had been treated with b-lactams shortly before the study. The PRP isolates from OR revealed a significant similarity of PFGE patterns and they belonged to the multiresistant pneumococcal clone Poland 23F-16. The single PRP isolate from FC represented another PFGE type.
Conclusions:
1. The carriage rate in DCC and OR was definitely higher than in FC;
2. The prior treatment with b-lactams correlated well with the nasopharyngeal carriage by PRP;
3. Around 80% of the isolates identified in DCC and OR belonged to the serotypes which are represented in the heptavalent pneumococcal conjugate vaccine;
4. Small closed communities, such as OR, create a high risk of the 'silent' clonal spread of pneumococcal strains with dangerous characteristics.

P1356 *Streptococcus pneumoniae* colonization in the pharyngeal flora of the elderly residents of a retirement-home in Ankara, Turkey

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Objectives: To determine the colonization rate of *Streptococcus pneumoniae* in the pharyngeal flora of institutionalized elderly people.
Methods: This descriptive study was held in one of the biggest retirement-homes in Ankara, Turkey in June 2002. We aimed to include all the elderly residents ($N = 207$) in that institution but due to some constraints 146 (70.5%) were reached. The data about demographic characteristics were gathered via a questionnaire implemented face-to-face interview by intern doctors; medical examinations were done and throat swab specimens were collected. The specimens were inoculated on 5% sheep blood agar. After incubation at 37°C in a candle jar for 48 h, alpha-hemolytic colonies were then subcultured on 5% blood agar and identified as *S. pneumoniae* if they were catalase negative, susceptible to optochin and soluble in bile salts.
Results: 62.3% of the study group was >75 years of age, 50.7% was female, and 68.5% was illiterate, literate or primary school graduate. More than two-thirds were widowed. Current smokers consisted 28.1% of the group. The most commonly stated complaints were artralgia, rheumatism, extremity pain, dyspnea, and dizziness. 13.6% stated that they had experienced fever within the last 2 months, and 13.1% within the last week. One-third of the elderly people was on cardiovascular medications and almost all of them indicated that they frequently used antibiotics. We could not detect a single strain of *S. pneumoniae* in the pharyngeal flora of the elderly people.
Conclusion: On the contrary of the expectations, no *S. pneumoniae* was detected in the study group. The season the study was performed and/or the low number of the people involved could explain this finding. The superfluous use of antibiotics could be another reason. Since the rate of colonization is seasonal and increases in mid-winter, it would be informative to repeat this study in winter. Although we could not detect pneumococci in the pharyngeal flora of the elderly people living in this closed society, vaccination of people older than 60-year-old could be accepted as one of the most important preventive measures as a public health policy with the fact of increasing emergence of penicillin-resistant pneumococci (30–40% intermediate resistance) in Turkey.

P1357 From prolonged febrile illness to fever of unknown origin: the challenge continues

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Objectives: Epidemiological changes and the ongoing expansion of the diagnostic armamentarium warrant a regular update of the spectrum of diseases that present as prolonged febrile illnesses.
Methods: We prospectively collected a series of 290 immunocompetent patients with a febrile illness (temperature >38.3°C) of uncertain etiology of >3 weeks duration, referred to our university hospital between 1990 and 1999. Patients were categorized in four groups according to the timing of diagnosis: early diagnosis (within three in-hospital days of three outpatient

visits), intermediate diagnosis (from day 4–7), late diagnosis (after day 7), and no diagnosis during index contact or follow-up.
Results: A final diagnosis was established early in 67 patients (23.1%), intermediate in 38 (13.1%), and late in 87 (30.0%). In the remaining 98 (33.8%), no diagnosis was made. The etiology of the fever remained obscure in 50 of 105 (47.6%) patients with episodic fever vs. in 48 of 185 (25.9%) patients with continuous fever ($P < 0.0005$). Among the 192 patients with a final diagnosis, noninfectious inflammatory diseases represented the most prevalent diagnostic category (35.4%), surpassing infections (29.7%), miscellaneous causes (19.8%), and malignancies (15.1%). Fourteen disorders (endocarditis, tuberculosis, Epstein–Barr or cytomegalovirus infectious mononucleosis, lymphoma, leukemia, adult-onset Still's disease, systemic lupus erythematosus, polymyalgia rheumatica/giant cell arthritis, sarcoidosis, Crohn's disease, subacute thyroiditis, habitual hyperthermia, and drug fever) accounted for over 59% of diagnoses, whether diagnosis was reached early, intermediate or late. Hematological malignancies made up 11.5% of diagnoses, but were responsible for 14 of the 24 (58.3%) fatalities related to the febrile illness. Three of the 80 patients discharged alive without diagnosis and for whom follow-up was available, died but the deaths were considered to be unrelated to the feverish illness.
Conclusions: Prolonged febrile illnesses remain a diagnostic challenge. Despite the technological progress of the late 20th century the origin of the fever remains elusive in many patients, especially in those with episodic fever. Noninfectious inflammatory diseases emerge as the most prevalent diagnostic category.

P1358 Rubella immunity status in pregnant women in Kuala Lumpur, Malaysia

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Background: In many developed countries, the incidence of rubella and congenital rubella syndrome (CRS) is considered to be negligible due to the availability of an effective vaccine. However, in Malaysia, CRS cases are occasionally seen every year. This will question the effectiveness of rubella vaccination programme incorporated in Malaysia's Expanded Programme on Immunisation (EPI). Currently, Malaysia practices Selective Rubella Immunisation Programme by vaccinating 12-year-old schoolgirls routinely since 1988 and unscheduled vaccinations for other women of childbearing age. Based on this, the eldest age for women who have undergone the routine rubella vaccination is 26 years old. Determining rubella immunity status among women of childbearing age is a good method to estimate the seriousness of rubella problem in Malaysia.
Objective: To determine the percentage of rubella immunity status among pregnant women attending antenatal clinics in National University of Malaysia Hospital in Kuala Lumpur, Malaysia.
Methods: The hospital database on rubella immunity was assessed retrospectively from August 2001 to June 2002. A prospective study of interviewed method as well as determination of rubella immunity by laboratory tests were carried out to pregnant women attending antenatal clinics in July 2002. Past history of rubella vaccination was specifically asked.
Results: A total of 414 women were included, of whom 134 women were interviewed. The rubella immunity status was 92.3% with highest percentage in the 15–26 age group (92.7%) and lowest in the 27–34 age group (88.9%). Among the interviewed women, 61.9% gave history of rubella vaccination (Group 1) and 23.8% were without history of vaccination (Group 2). Six percent of Group 1 and 9.3% of Group 2 did not have protective rubella antibodies. The antibody levels in immune women in Group 2 are significantly higher than immune women in Group 1 indicating the possibility of natural rubella immunity.
Conclusions: The high percentage of immune women in Group 2 may indicate high infection rate of rubella in nonimmune individuals in the general population. This may explain the sporadic incidence of CRS cases in Malaysia. A small percentage of nonimmune women in the 15–26 age group may indicate primary or secondary vaccine failure, which is not looked into seriously. This can also contribute to the incidence of CRS in Malaysia.

P1359 Increased incidence of tick-borne encephalitis in the 1990s in Central and Eastern Europe

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Objectives: Epidemiological analysis of tick-borne encephalitis (TBE) occurrence in Europe and an attempt to elucidate this phenomenon.
Methods: Collection and evaluation of epidemiological, socioeconomic, parasitological, zoological and meteorological data.
Results: A sharp increase of TBE incidence occurred in nineties in 11 Central and Eastern European countries. In 10 of them, maximum incidence was recorded in years 1993–1997 and has remained high since, with some slight fluctuations. The situation is attributable to more abundant occurrence of TBE virus vector *Ixodes ricinus* tick. High density of tick populations is due to modified climatic conditions in the last decade namely in autumn resulting in their longer host-seeking activity period – up to November and subsequent high occurrence of ticks in following season. Mild winter seasons in the nineties have facilitated a more successful wintering of animal hosts, mainly small terrestrial mammals. Favorable climate conditions in early spring time (in the year 2002 the ticks were active at the end of February in the Czech Republic) resulted in longer period of tick activity a resulted in first cases of TBE disease in March. This extension of TBE incidence in early spring and late autumn was observed throughout that period. Shift of *I. ricinus* distribution to higher altitude was found Czech mountain areas. Simultaneously the shift of the tick *I. ricinus* and TBE into higher geographic latitudes was described by Swedish and Danish authors. Behavioral and socioeconomic aspects don't play any role in described increase of TBE incidence.
Conclusions: Increased TBE incidence in nineties is attributable to climate changes causing longer period of *I. ricinus* activity and favorable conditions for wintering of this tick species.
Acknowledgement: This study was supported by the project Climate change and Adaptation Strategies for Human Health (cCASHh), Contract Nr.EVK2-2000–00670.

P1360 Surveillance study of the incidence of tuberculosis and hepatitis B in a high-risk group of slum-dwelling female rag pickers in Jaipur, India

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Introduction: Rag pickers constitute the lowest socioeconomic group and women in a male dominating social structure are further disadvantaged. Fifteen percent of the municipal solid waste is lifted by rag pickers; mostly from rubbish dumps located near hospitals and include biomedical waste. Hazards of occupation, overcrowded slum dwellings, poor nutrition, neglect of health and hygiene, and lack of medical makes them a high-risk target for infectious diseases.
Objective: Surveillance study to establish the incidence of hepatitis B and tuberculosis in this high-risk target group of slum-dwelling female rag pickers in the city of Jaipur and using this base line data to permit Jaipur Municipal Corporation to consider executive policy changes in the welfare of rag pickers.
Methods: A total of 1500 slum dwelling female rag pickers in the age group 18–60 years were identified as the target group from the list provided by the Jaipur Municipal Corporation. They were screened over a period of 6 months for tuberculosis using Ziehl–Nielsen staining (ZN) of sputum for acid–alcohol fast bacilli (AAFB) and hepatitis B using a strip ELISA method on serum to detect HBsAg.
Results: Sputum and serum samples from 1500 women were screened. Sputa from 12 women (0.8%) were found positive (1+) for AAFB while 82 (5.46%) sera tested positive for hepatitis B (HBsAg by strip ELISA).
Conclusion: Scavenging through biomedical waste dumped near hospitals and possibility of sharp injuries from disposed needles are possible risk factors for hepatitis B and to a lesser extent for HIV. Poor socio-economic conditions of living, overcrowding, lack of adequate hygiene, nutrition, and medical care

predisposes the rag pickers to tuberculosis and other infectious diseases. Data from this study provided a yardstick for Jaipur Municipal Corporation to bring about major policy changes in the welfare of this high-risk group, which is discussed.

P1361 Rickettsioses: the analysis of 42 cases diagnosed over 14 years

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Objective: The causative agent of Marseille fever which is an endemic rickettsiosis for our country is *R. conorii*. Forty-two rickettsioses cases which were hospitalized at Haseki Education and Research Hospital during the period 1989–2002 have been examined according to their epidemiological, clinical and laboratory properties.
Methods: The patients who applied to the hospital with fever and rash had been diagnosed according to the patient's history, the property of the rash and Weil–Felix test. The Weil–Felix test which was studied by OX-2 and OX-19 antigens was found to be positive at 1/80 and 1/160 titers. Almost in all the patients 'tache noire' was detected. Twenty patients were female and 22 were male. Their ages ranged between 20 and 70 and the average was 40. When the application date is observed, it has a peak in August for female patients and males were hospitalized through July, August, and September.
Result: The hospitalization duration ranged between 1 and 38 days and the mean was 5–10 days. Doxycycline had been used for therapy for 7–10 days. The patient who was hospitalized for 38 days died, all the others recovered.
Conclusion: Although the incidence of rickettsioses in our country is low, it must be remembered for the differential diagnosis, especially in patients who may contact with ticks and who apply with fever and rash.

P1362 The problem of group A beta-hemolytic streptococcal infections and complications in ambulatory outpatient clinics in Yemen

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Objectives: To show the increasing rates of streptococcal and poststreptococcal medical problems in the daily outpatient practice in a hospital in Yemen. To show the rates of cardiac and articular involvement. To reveal the level of ampicillin resistance.
Methods: Study group of 394 patients of various age groups interviewed in an outpatient clinic in a period of 8 months (April to November 2002). Selection made on clinical basis (complaining of arthralgia, arthritis, fever, having tonsillitis or tonsillar enlargement, and/or cardiac complaints). All the study patients were investigated for Anti-Streptolysin O titer, throat swab culture and sensitivity test, C-reactive protein, erythrocyte sedimentation rate and total leukocyte count. Patients with cardiac complaint also had electrocardiograms, chest X-rays and echocardiograms.
Results: Evidence of group A beta-hemolytic streptococcal infection was found in the study group in 69.04% (272/394) by elevated ASO titers and in 51.27% (202/394) by positive throat swabs. This was more marked during cold months November (ASO 75.86% and cultures 56.32%) and October (69.35% and 59.68%). Rates of cardiac complications were high (37.82%). Arthralgia found in 75.13% and arthritis in 42.64%. High rates of ampicillin resistance noticed in streptococcal isolates (64.36%).
Conclusions: Group A beta-hemolytic streptococcal infections and postinfection complications forms a major medical problem in Yemeni population. It causes a great morbidity and poses a big challenge to the national and international health programs. This is especially important due to the development of antibiotic resistance resulting from antibiotic abuse, and neglect of early and adequate treatment and prophylaxis of affected children and adolescents.

P1363 Whipple disease: diagnosed 22 years after symptom onset

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A 58-year-old man was admitted to our hospital due to anemia, weight loss, migratory polyarthralgias with inflammatory sings and diarrhea of various years of evolution with hyperpigmentation of skin. The patient had been known to have multiple episodes of athralgias, intermittent fever and bowel disturbances along with multiple admissions to the hospital dating back almost 22 years. His past medical history was consistent with febrile migratory polyarthralgias 22 years ago, hemorrhagic inflammatory bowel disease 18 years ago, seronegative arthritis 5 years ago due to he had received steroid therapy. Physical examination of the patient revealed hyperpigmented, dry skin, the liver and spleen were moderately enlarged and excessive weight loss. Laboratory tests revealed a hemoglobin concentration of 9.7 g/dL (MCV 73 fL) with normal leukocyte and platelet counts. Computed tomography of the abdomen and chest showed no abnormal lesions. Gastroduodenoscopy disclosed slight edema in the second portion of the duodenum. Biopsy specimens from the distal duodenum revealed histopathologic features of Whipple disease, including periodic acid Schiff (PAS)-positive macrophages. The patient was placed on antibiotic therapy with vibramycin and follow-up after 3 months the patient reported less fatigue. We report this case in which the diagnosis was made 22 years after his initial symptom. According to literature, there is another one case in whom the diagnosis was made 36 years later. Whipple disease is a very rare multisystem disorder caused by *T. whippeli*. It is difficult to diagnose unless there is a high index of suspicion. Key to the definitive diagnosis of Whipple disease is mucosal biopsy specimens from the distal duodenum and jejunum and the diagnosis of Whipple disease averaged 2–9 years after onset of symptoms.

PCR and parasites

P1364 Detection of *Pentatrichomonas hominis* DNA by PCR in biological specimens

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Objectives: *Pentatrichomonas hominis* is an human trichomonad that inhabits the digestive tract. It is not a pathogen in the intestinal tract but has been associated with liver abscesses and a case of pulmonary trichomoniasis has been described. *P. hominis* and *T. vaginalis* do grow in the same culture media and are indistinguishable by light microscopy. To differentiate these two human trichomonads we aimed to develop a Polymerase Chain Reaction (PCR) assay for detection of *P. hominis* in biological specimens.
Methods: Two primer pairs, Th3/Th5 and Th4/Th5, were used to amplify, respectively, 339 bp and 785 bp from an unique region of the 16S-like ribosomale RNA gene of *P. hominis*. DNA was extracted with the use of the QIAamp DNA Mini Kit (Qiagen). The target DNA was amplified in a Perkin-Elmer, Gene Amp 9600 thermal cycler. The amplified PCR product was detected by examination of ethidium-bromide stained agarose gel. Presence of DNA and PCR inhibition was checked for the human specimens by the parallel amplification of human beta-2 microglobulin and/or *Escherichia coli* DNA.
Results: The results obtained with the Th3/Th5 and the Th4/Th5 primer pair were identical. No amplification was obtained with DNA extracts of pure cultures of *G. vaginalis*, *N. gonorrhoeae*, *N. lactamica*, *S. aureus*, *E. faecalis*, *Escherichia coli*, *C. albicans*, *T. vaginalis*, *T. gallinae*, *T. foetus*, *T. gallinarum*, *T. tenax*, *D. fragilis*, and *G. lamblia*. Reproducible amplifications of DNA from 3 *P. hominis* reference strains were obtained. DNA diluted samples with a final concentration of 1 *P. hominis* organism were amplified, and gave positive results. A total of 113 feces extracts were amplified, no positive results were obtained. The following pathogens were detected in the feces specimens using microscopy and bacteria culture: *Entamoeba coli*, *E. histolytica/dispar*, *E. nana*, *I. butschlii*, *T. trichuria*, *Campylobacter jejuni*, *Ancylostomatoidae*, *S. mansoni*. Thousand vaginal specimens were tested by PCR for *T. vaginalis* and

P. hominis. DNA targets of *P. hominis* were amplified by both primer sets in two vaginal specimens, while two PCR assays with different DNA targets for *T. vaginalis* gave no PCR product.
Conclusion: We developed a highly sensitive and specific PCR assay for the detection of *P. hominis*. *P. hominis* DNA targets were detected by PCR in two vaginal specimens. The presence of *P. hominis* in vaginal specimens has so far not been described in the literature and needs to be further explored.

P1365 Ocular toxoplasmosis – two case reports

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Goals: Describe two clinical cases of ocular toxoplasmosis in order to understand and emphasize the importance of polymerase chain reaction (PCR) in the management of ophthalmologic patients.
Case reports: The first clinical case refers to a 69-year-old man, with no relevant clinical history, with sudden onset of blurred vision and photophobia, preceded 1 week before by bilateral red eye with spontaneous resolution. The patient presented with right eye visual acuity of 40/200 and left eye (LE) visual acuity of 140/200, LE defect, no biomicroscopic abnormalities, normal intraocular pressure, localized cotton-wool spots obscuring retinal vessels and diffuse vitritis at fundoscopy in both eyes. The second clinical case refers to a 23-year-old man, with no relevant clinical history, with sudden onset of blurred vision and photophobia of the LE. The patient presented with right eye visual acuity of 200/200 and LE VA of 20/200, no abnormal pupillary reflexes or biomicroscopic abnormalities, normal intraocular pressure and localized cotton-wool spots obscuring retinal vessels at fundoscopy in the LE.
Methods: Both patients underwent complete ophthalmologic study, including campimetry, fluorescein angiography, color vision tests and complete serologic analysis. In the first case, a lumbar punction was performed. In the second patient we performed anterior chamber punction as well as vitreous punction. The samples were also tested for *Toxoplasma gondii* DNA using primers from the *B1* gene in a PCR.
Results: The angiogram revealed active lesions of retinochoroiditis with associated arteritis. The liquor polymerase chain reaction was positive for *T. gondii* DNA in the first patient. In the second patient the vitreous polymerase chain reaction was also positive for *T. gondii* DNA. Flow citometry was normal in both cases, revealing immunocompetency.
Conclusion: The patients underwent triple drug therapy with pyrimethamine, sulfadiazine and prednisone for 6 weeks. Anatomical and functional improvement ocurred with resolution of diffuse vitritis. Prednisone was discontinued 2 weeks before antitoxoplasmic agents. Although traditionally based on clinical history, fundus examination and serologic evidences, modern diagnosis methods are getting increasingly more important for the correct management of ophthalmological patients. Nevertheless, PCR is only capable of detecting *T. gondii* DNA in aqueous or vitreous samples in only one third of patients with ocular toxoplasmosis.

P1366 Development of a multiplex real-time PCR assay to detect the four species of human *Plasmodium* in blood

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Objective: These last decades, tourism and population migrations have caused an increase of malaria cases reported in immigrants and travelers. Although microscopic examination of blood smears remains the gold standard in diagnosis, this method as well as antigen detection suffers from insufficient sensitivity in case of low parasitemia. In order to improve diagnosis, we have developed a rapid and user-friendly multiplex real-time PCR.
Materials and methods: One set of generic primers was designed in a highly conserved region of the 18S rRNA gene of Plasmodium genus, internally polymorphic enough to allow selection of four species-specific probes for *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*. Positive controls and standard curves were generated with four plasmids, containing each cloned species-specific amplified sequence. Moreover, a Plasmodium genus-specific probe was designed for screening purpose. So far a retrospective evaluation of this real-time PCR was carried out blindly on 43 clinical samples (43 patients). For PCR, DNA from 200 µL of EDTA-treated blood stored at −20°C was purified with the MagnaPure system (Roche). Five microliter DNA was used in a 25 µL reaction containing primers, probe and real-time PCR master mix (ABI).
Results: Real-time PCR with species-specific probes was able to detect 1 plasmid copy of *P. falciparum*, vivax, malariae and ovale in a highly specific manner. The same sensitivity was achieved for all species using real-time PCR with the Plasmodium genus probe. Microscopy (mi), antigen detection (ag) and real-time PCR (PCR) for the 43 blood samples had an overall agreement of 86%. Fifteen samples were positive and 22 were negative with the 3 tests. Six specimens (3 mi−/ag+/PCR+ 1 mi+/ag−/PCR+ 2 mi+/ag−/PCR−) were discordant and are being reevaluated on the basis of clinical, molecular and sequencing data to assess their true value. However, meanwhile these six specimens are considered true positive/negative when at least two out of three tests results are positive/negative, resulting in an overall PCR sensitivity and specificity of 100%.
Conclusion: Real-time PCR is a rapid and sensitive method for detecting human Plasmodium. With sensitivity of microscopy being about 500–50 parasites per microliter (thick blood film), real-time PCR is potentially 100-fold superior to microscopy. Further validation on patient samples is needed to assess the clinical settings which will benefit from this diagnostic method.

P1367 A screening for oral trichomonad infection in HIV patients by PCR-RFLP

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Objectives: Oral lesions, advanced caries and other oral manifestations often occur in HIV patients. The aim of the study was searching for oral trichomonads in these patients. PCR-RFLP was performed for detection and species differentiation of oral parasites.
Methods: Fifty-four homeless HIV patients (18 females and 36 males at the age of 20–54) were examined. All of them were addicted to intravenous drugs for at least 6 years. Thirty-eight patients were treated with antibiotics and antifungals. The materials taken from the patients were: saliva, smears and spittle. For PCR amplification a pair of primers specific for ITS 1–5.8SrRNA – ITS 2 region was designed; for restriction analysis two endonucleases: Dde I and Msp I were used.
Results: Three patients (males at the age of 25, 27 and 44) were infected with oral trichomonads. The identification of species by PCR and digestion with Dde I and Msp I showed the trichomonads belonged to *Trichomonas tenax* (confirmed by sequencing).
Conclusions: The infection of healthy humans with *Trichomonas tenax* is 10–12%, but in patients with periodontitis or gingivitis – about 50%. Only 5.5% HIV patients were infected with *Trichomonas tenax*. Infection with *T. tenax* in two young patients is unusual in comparison with HIV negative persons.

Rapid diagnosis of respiratory pathogens

P1368 Detection of *Mycoplasma pneumoniae* in respiratory samples by real-time PCR using an inhibition control

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Objectives: To evaluate a real-time PCR assay using two adjacent fluorescent probes in a Lightcycler instrument (Roche diagnostics, Belgium) for detection of the *Mycoplasma pneumoniae P1* gene, using an internal control that can be amplified by the same primers but detected by different probes to monitor inhibition in each sample.
Methods: Primers were chosen complementary to part of the *M. pneumoniae P1* gene. An internal control that can be amplified by the same primer pair was constructed to monitor inhibition in each reaction. Two sets of adjacent fluorescent probes were chosen complementary to the *M. pneumoniae* target and to the internal control target for real-time detection in the Lightcycler.

The use of different labels for the probe sets allows their simultaneous detection by dual colour detection. After specificity and sensitivity testing of the assay, DNA isolated from 115 respiratory samples from patients enrolled in a study on lower respiratory tract infections ($n = 80$) or community acquired pneumonia ($n = 35$) was analyzed with the Lightcycler assay. The samples had previously been tested with an in house PCR assay targeting the same part of the *P1* gene.

Results: The Lightcycler assay proved to be highly specific, reacting only with DNA extracted from *M. pneumoniae*. The assay could detect purified DNA extracted from as little as 500 colour changing units (ccu) of *M. pneumoniae* per ml (1 ccu is estimated to correspond to 10–100 organisms). Upon analysis of the 115 respiratory specimens, there was 100% agreement between the real-time and the conventional assays (86 specimens were negative, 29 were positive), but the real-time PCR proved to be highly superior in speed with a much lower risk of false positives by laboratory contamination. Two of the 86 *M. pneumoniae* negative specimens showed no amplification of the internal control. Upon 1/5 dilution of the samples the inhibition was eliminated.

Conclusions: The Lightcycler assay for *M. pneumoniae* detection proved to be a very sensitive and specific diagnostic tool, allowing detection of *M. pneumoniae* DNA in respiratory samples within two hours after receipt of the sample. Moreover, the use of an internal control amplified by the same primers but detected by different probes, proved to be useful to avoid false negative results.

P1369 Development of conventional NASBA for the detection of *Bordetella pertussis* in respiratory specimens

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Introduction: *Bordetella pertussis* is a major etiologic agent of prolonged cough during childhood in unvaccinated populations. During the past 10 years, resurgence of pertussis has been seen in several countries with high vaccination coverage. Therefore, rapid diagnosis is crucial to eradicate the source of infection and to treatment of each contact early, particularly nonvaccinated infants in whom pertussis might present as a life-threatening disease.

Objectives: The aim of the study was to develop a NASBA assay for the detection of *B. pertussis* in respiratory specimens based on NASBA amplification of a 16S rRNA target sequence using the NucliSens Basic Kit® (bioMérieux).

Methods: Oligonucleotide primers were derived from the *B. pertussis* 16S rRNA. The assay was developed using the NucliSens Basic Kit, that includes standardized reagents for nucleic acid extraction, RNA amplification and electrochemiluminescence (ECL) detection. Specificity was established on a panel of bacterial strains. The analytical sensitivity of the assay was determined through testing dilutions of different *B. pertussis* strains. Serial dilutions of *B. pertussis* were added to pools of respiratory specimens and treated with protease before extraction. Subsequently, a limited number of *B. pertussis* positive and negative clinical specimens were analyzed.

Results: Using the *B. pertussis* 16S rRNA NASBA primers in combination with the generic NucliSens Basic Kit ECL probe and a biotin capture probe, positive results were obtained with nucleic acid extracts from *B. pertussis* strains, *B. parapertussis* strains, *B. bronchiseptica* strains and with a *B. holmesii* strain but with none of the other organisms. The primers enabled detection of as little as 1–10 *B. pertussis* CFU. Applied on clinical specimens spiked with *B. pertussis*, the sensitivity varied between 10 and 100.000 CFU depending on the kind of respiratory specimen. Four out of six clinical specimens found to be *B. pertussis* positive by the Belgian Bordetella Reference Centre were also positive in the NASBA assay. In the other two samples RNA degradation had occurred. None of 100 *B. pertussis* culture negative clinical specimens were positive by real time PCR. However, two specimens were found to be *Bordetella* positive by NASBA.

Conclusions: The NucliSens Basic Kit® could become a fast, useful and user-friendly device for the development of diagnostic tools like the NASBA-based assay for the detection of *Bordetella* species in respiratory specimens.

P1370 Detection of *Mycoplasma pneumoniae* in respiratory specimens using real-time NASBA

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Introduction: *Mycoplasma pneumoniae* is a common etiologic agent of respiratory tract infections in humans, responsible for 15–20% of all cases of pneumonia and a wide range of mild to serious extrapulmonary complications. In the past, diagnosis of infection by this organism was frequently based on serology because culture is slow and insensitive. Therefore, real-time NASBA (nucleic acid sequence-based amplification) might offer an alternative.

Objectives: The aim of the study was to develop a real-time NASBA assay for the detection of *M. pneumoniae* in respiratory specimens based on real time NASBA amplification of a 16S rRNA target sequence using the NucliSens Basic Kit® (bioMérieux).

Methods: Oligonucleotide primers were derived from the M. pneumoniae 16S rRNA. The assay was developed using the NucliSens Basic Kit, including standardised reagents for nucleic acid extraction, RNA amplification and electrochemiluminescence (ECL) detection. For real-time detection, a molecular beacon was used. Specificity was established on a panel of bacterial strains. The analytical sensitivity of the assay was determined through testing of dilutions of a *M. pneumoniae* reference strain, PI1428. Serial dilutions of *M. pneumoniae* PI1428 were added to pools of respiratory specimens and treated with protease before extraction. Subsequently, a limited number of *M. pneumoniae* positive and negative clinical specimens were analyzed. The results obtained with the real time *M. pneumoniae* 16S rRNA assay were compared with the results obtained by conventional NASBA with ECL end-point detection.

Results: Specific detection of the 16S rRNA-derived amplicons was achieved: all *M. pneumoniae* strains tested positive, whereas other bacterial strains tested negative. The sensitivity of the NASBA assay was five colour changing units (CCU) of *M. pneumoniae*. In spiked throat swabs, nasopharyngeal aspirates, bronchoalveolar lavages and sputum, the sensitivity of the NASBA assay was 5–50 CCU of *M. pneumoniae*. The sensitivity of the real time NASBA assay was comparable to that of conventional NASBA. Seventeen clinical specimens positive for *M. pneumoniae* by PCR, were also positive by conventional NASBA but one specimen was negative by real time NASBA.

Conclusions: The NucliSens Basic Kit combined with real-time detection appeared to be a fast, useful and user-friendly diagnostic tool for the development of a NASBA-based assay for the detection of *M. pneumoniae* in respiratory specimens.

P1371 Development of a PCR assay and typing method for invasive pneumococcal disease based on the detection of the *pneumolysin* gene

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Objectives: Pneumococcal disease is a leading cause of mortality and morbidity worldwide. It is primarily caused by *Streptococcus pneumoniae*. *S. pneumoniae* produces *pneumolysin* (*ply*) as a virulence factor which exerts its effect on human host cells. A PCR assay has been developed for the detection of the *ply* gene and nonculture laboratory conformation of invasive pneumococcal disease. It has been reported that the *ply* gene varies between different serotypes of *S. pneumoniae*. Thus the *ply* genes from the 23 serotypes most commonly causing invasive disease were sequenced to explore a potential sequence-based typing method.

Methods: A nested PCR test was developed, as the concentration of DNA in clinical samples can be low. Primers were designed to amplify the 1.6 kb *ply* gene. Clinical samples were tested for the presence of the *ply* gene using the nested PCR. Sensitivity and specificity were determined. The *ply* gene from 23 most common serotypes was sequenced and analyzed.

Results: Five hundred and twenty clinical samples were used to evaluate the PCR method (208 CSF, 247 serum, 65 whole blood). Seventeen of the clinical samples were confirmed pneumococcal antigen positive, 12 of these were positive using this PCR test. In total, 15 PCR positives were detected that had not previously been tested for pneumococcal infection. Specificity was assessed using organisms that may be found in the serum and CSF ($n = 48$), of these organisms 4 were positive (*S. parasangnis*, *S. oralis* and two *Neisseria lactamica*). The initial PCR detected confluent growth, adding the nested stage increased sensitivity 10-fold (158 cfu detected). A consensus sequence for each serotype was prepared using the sequence data. The 23 consensus sequences were aligned to show sequence variation. There was insignificant variation between the consensus serotypes, thus it was not possible to design oligonucleotide primers that would specifically amplify individual serotypes of *S. pneumoniae*.

Conclusion: The PCR developed will detect the *ply* gene in clinical samples although, as sensitivity is low, it may not detect all positive samples. This study demonstrated there are no significant differences between *ply* genes from

different pneumococcal serotypes and therefore it is not possible to develop a serotype specific assay for the detection of *ply* in clinical samples.

P1372 Detection of macrolide resistance genes in *Streptococcus pneumoniae* using a rapid method

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Genoa, I

Objectives: Macrolide resistance in streptococci occurs predominantly by two mechanisms: target modification or efflux. The first results in coresistance to macrolide, lincosamide and streptogramin B (MLSB phenotype) and it is mediated by two classes of methylase genes, the conventional *ermAM* (*ermB*) determinant and the recently described ermTR determinant. The second, conferring low macrolide MICs and retain susceptibility to clindamycin (M phenotype), is mediated by *mefA* gene. The aim of this study was to develop a rapid large-scale screening of macrolide-resistant genotypes in isolates of *S. pneumoniae*.
Methods: Fifty strains of clinical isolates of erythromycin-resistant (40 belonging to MLSB phenotype and 10 belonging to M phenotype) and 10 erythromycin-susceptible *S. pneumoniae* strains have been tested. MICs for erythromycin and clindamycin were determined by the microdilution method as suggested by the NCCLS (2002). The genes were amplified by a multiplex rapid cycle PCR, using biotinilated primers. The products were added to streptoavidin-coated microwell and were incubated with fluorescinated probes, specific for genes *ermB*, *mefA* and *ermTR*. Detection of amplified products was carried out using a modification of the method described by Farrell et al. JAC, 2001. The products were incubated with antifluorescin antibody conjugated with peroxidase and then *o*-phenylenediamine (OPD) was added to each well. Absorbances (450 nm) were read by spectrophotometer.
Results: The method proved to be very rapid, results were obtained within 4 hours. As we expected, according to MIC phenotypes, 40 *S. pneumoniae* isolates characterized by MLSB phenotype were positive for *ermB*. The remaining 10 strains showing an M phenotype reacted with the *mefA* probe. All macrolide-susceptible isolates tested were negative for the mechanisms under investigation. Strains having dual mechanisms of macrolide resistance (*ermB* + *mefA*) were not found. Using this method, concordance between MIC phenotypes and genotypes was 100%.
Conclusions: The method reported in this study allows to standardize testing of large number of isolates and to discriminate within different genotypes with an only multiplex PCR reaction. It allows the analysis of a large number of specimens (96 for each microwell) within in short time. In addition, the use of an oligonucleotide probe adds specificity to the detection, which increases the reliability of the results.

P1373 Comparison of magnetic and conventional Boom RNA extraction using respiratory samples

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Introduction: The use of nucleic acid amplification techniques for the detection of infectious agents in clinical specimens continues to expand, and these techniques promise to play an increasing role in the diagnostic laboratory. While a great deal has been published on amplification protocols, less information is available addressing specimen processing for optimal RNA recovery prior to amplification.
Objectives: A new sample preparation system that combines the advantages of the Boom method for RNA extraction from clinical samples with the convenience of the magnetic bead technology was evaluated and compared with conventional RNA extraction using the NucliSens Basic Kit® (bioMérieux).
Methods: Sputum samples were pretreated with protease, aliquoted and the appropriate lysis buffer was added. Conventional RNA extraction was done using the NucliSens Basic Kit according to the instructions of the manufacturer. For magnetic RNA extraction, the samples were first incubated for 10 min at 60°C and then centrifuged for 15 min at 13000 rpm. Then the supernatants was added to the magnetic silica, centrifuged, washed twice with wash buffer 1, wash buffer 2, followed by elution of the nucleic acids. U1A mRNA was amplified by NASBA and the amplicons were detected by electrochemiluminescence (NucliSens Basic Kit). Finally, a limited number of *L. pneumophila*, *M. pneumoniae* and *B. pertussis* specimens were analyzed by both extraction methods. The results obtained with both extraction procedures were compared with each other.
Results: Conventional Boom extraction resulted in 12/20 U1A mRNA positive samples whereas magnetic extraction yielded 15/20 positive samples. On the undiluted extracts all 15, and only 8/20 positive results were obtained after magnetic and conventional extraction, respectively. Usually, at least 4-fold higher ECL-counts were found for extracts obtained by magnetic extraction. Five *L. pneumophila* positive lung biopsy specimens, 1 *B. pertussis* and 1 *M. pneumoniae* positive sputum, 1 *M. pneumoniae* positive gargle specimen, and 1 *M. pneumoniae* throat swab were positive by both extraction methods. Again, higher ECL counts were generally found for extracts obtained by magnetic extraction.
Conclusions: The NucliSens Basic Kit® combined with magnetic RNA extraction could become a fast, useful and user-friendly diagnostic tool for a NASBA-based assay for the detection of respiratory pathogens in clinical specimens.

P1374 PCR for diagnosis of *Chlamydophila pneumoniae* from respiratory specimens

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Objectives: *Chlamydophila pneumoniae* is an important respiratory pathogen in both adults, with and without COPD, and children and has recently been associated with atherosclerosis and several other chronic diseases. The organism can be detected by antibody detection methods or by using PCR. Published detection rates of *C. pneumoniae* in respiratory diseases are inconsistent and vary from 5–33% in chronic obstructive pulmonary disease (COPD). We have used standardized PCR assays, using three previously described primers, to examine an unselected group of 750 sputa submitted sequentially to a routine diagnostic laboratory to determine in two stages firstly the prevalence of *C. pneumoniae* DNA in a sample of sputa submitted for examination to laboratories in the UK and whether the rate of positivity varies between different clinical groups and secondly is the amount of chlamydial DNA independent of the number of White blood cells in the sputum.
Methods: *Chlamydophila pneumoniae* was detected by DNA Amplification of the 16S rRNA Gene and quantitation of the amount of *C. pneumoniae* DNA in the sputum measured using the fluorescent dye-labeled TaqMan probe-based system and oligonucleotide primers and probes designed to target two variable domains of the *ompA* gene, VD2 and VD4.
Results: Using DNA extracted from sputa we have found that 28% of this unselected population are positive for *C. pnemoniae* by routine PCR. We have compared these results with two real-time PCR assays specific for *C. pneumoniae* and will present data on:
1. The correlation of real time and standard PCR methodologies
2. The quantitation of *C. pneumoniae* DNA.
3. The correlation of PCR, bacterial culture and clinical features of infection.

Conclusion: *C. pneumoniae* DNA is found in 28% of all sputa submitted to a routine diagnostic laboratory. Further work is required to ascertain the significance of this in different clinical conditions.

P1375 Detection of *Chlamydia pneumoniae* in respiratory tract specimens by PCR: evaluation of methodology

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Objective: Infection of the respiratory tract with *C. pneumoniae* is reported with increasing frequency. Gold standard for diagnosis of this infection is the microimmunofluorescence (MIF) assay, an expensive method with long turnaround time. This study evaluated three DNA extraction methods and a PCR assay using respiratory tract specimens from patients with acute lower respiratory tract infection (ALRTI).
Materials and methods: Sputum or BAL specimens and acute and convalescent serum samples from 95 hospitalized patients with ALRTI were tested retrospectively. A species-specific MIF assay (MRL, USA) was used for serological diagnosis. Respiratory samples were subdivided in three parts and DNA was extracted by proteinase K treatment, NucliSens (Organon Teknika, Belgium) and IsoQuick (Orca Research, USA). PCR testing was performed with a nested protocol amplifying part of the *ompA* gene. Inter- and intra-assay PCR reproducibility was assessed by repeat testing of PCR-positive samples.

Results: By MIF testing, 56 patients demonstrated acute infection with *C. pneumoniae* (1 IgG seroconversion, 3 a four-fold increase of IgG titers, 5 IgM positivity and 47 a significant IgG titer (≥1/512)) and 39 were MIF-negative. PCR sensitivity by any extraction method was 46%: 26 out of 56 respiratory samples originating from MIF-positive patients were PCR-positive. Twenty-five samples were positive by proteinase K treatment, 5 by IsoQuick and 2 by NucliSens (sensitivities 45, 10 and 4%, respectively). Twenty samples were positive only after proteinase K treatment. None of the 39 samples obtained from MIF-negative patients was PCR-positive by any extraction method (specificity 100%). Twenty-three PCR positive samples (extracted by proteinase K) were tested repeatedly (3–5 times) on different days. Overall, 57 out of 81 PCR tests revealed a positive result (PCR interassay reproducibility 70%). Only 10 samples (44%) consistently reproduced a positive result. Ten PCR-positive samples (extracted by proteinase K) were retested, each sample four times within the same PCR run. There were 26 PCR-positive results out of 40 expected (PCR intra-assay reproducibility 65%).
Conclusions: PCR assay sensitivity differs greatly between DNA extraction methods; best results are achieved with proteinase K. Although its specificity is excellent, PCR testing lacks interassay and intra-assay reproducibility. Diagnosis of respiratory infection with *C. pneumoniae* by PCR is problematic.

P1376 Development and application of a multiplex real-time PCR for diagnosis of *Mycoplasma pneumoniae, Chlamydia pneumoniae* and *Bordetella pertussis*

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Objectives: To develop a multiplex real-time PCR for clinical diagnosis of *Mycoplasma pneumoniae* (MP), *Chlamydia pneumonia* (CP) and *Bordetella pertussis* (BP) with an internal control.
Methods: Monoplex real-time PCR assays using molecular beacons as probes were developed for each pathogen. The molecular beacons were labelled with FAM (MP), HEX (CP), Texas RED (BP) so that discrimination in a single tube could be determined in one multiplex. All assays were first optimised in the monoplex assays and thereafter into one 4-target-multiplex reaction. The multiplex included an internal control reaction (phocine herpes virus) which was also detected by a labeled probe (Cy5). Standards of the bacteria were obtained by achieving a titer by colour changing units (CCU)/mL, inclusion forming units (IFU)/mL and Mcfarland turbimetric standards for MP, CP and BP, respectively. The assay was evaluated using samples from 93 patients with respiratory symptoms requesting investigation for at least one of the pathogens. All samples were tested by both the conventional diagnostic methods (serology or culture) and by means of the multiplex reaction. Positive results by real-time PCR only were confirmed by an alternate conventional PCR.
Results: The same limit of sensitivity was seen in the monoplex reactions and multiplex reaction, in that 50 CCU/mL, 0.049 IFU/mL, 100 CFU/mL was detected for MP, CP and BP, respectively. The specificity of the monoplex and multiplex was also the same. No other respiratory pathogens were detected by the assays except that BP assay also detected *B. holmesii* in both monoplex and multiplex formats. In the clinical testing 9/93, 0/93 and 6/93 were detected by conventional methods and 12/93, 0/93 and 15/93 were detected by the multiplex PCR for MP, CP and BP, respectively. Of these two extra positives were obtained which were not suspected by the clinician.
Conclusion: The Multiplex real-time PCR for MP, CP and BP provides sensitive and specific diagnosis of these respiratory pathogens, which are difficult to culture. The multiplex assay is suitable for diagnostic use and may improve patient diagnosis and management.

P1377 Qualitative and quantitative detection of *Chlamydia pneumoniae* DNA in cerebrospinal fluid

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Objectives: A diagnostic test for the detection and quantitation of *Chlamydia pneumoniae* DNA in cerebrospinal fluid (CFS) is needed because of the association of *C. pneumoniae* with a number of neurological disorders including multiple sclerosis (MS).
Methods: We developed and validated a qualitative colorimetric microtiter plate-based PCR-enzyme linked amplification and hybridization assay (MTP-PCR) and a real-time quantitative PCR assay (TaqMan) for detection of *C. pneumoniae* DNA in CSF specimens from MS patients and controls. The two methods were evaluated in comparison to a touchdown nested PCR (n-PCR) assay on a total of 137 CSF specimens.
Results: The sensitivity, specificity, and concordance of the MTP-PCR assay were 88.5, 93.2, and 90.5%, respectively. MTP-PCR presented a significantly higher sensitivity in MS patients ($P = 0.008$) and a higher specificity in other neurological diseases ($P = 0.018$). Test reproducibility of the MTP-PCR assay was statistically related to the volumes of extract DNA included in the test ($P = 0.033$); a high volume, which was equivalent to 0.1 mL of CSF per reaction, yielded a concordance of 96.8% between two medical technologists running the test at different times. The TaqMan quantitative PCR assay detected 26 of 59 (44.1%) CSF specimens that tested positive by both MTP-PCR and n-PCR qualitative assays. None of the CSF specimens that were negative by the two qualitative PCR methods were detected by the TaqMan quantitative PCR. MTP-PCR assay detected a minimum of 25 copies/mL *C. pneumoniae* DNA in plasmid-spiked CSF, which was at least 10 times more sensitive than TaqMan.
Conclusion: These data indicated that the MTP-PCR assay possessed an equivalent sensitivity to nested procedures for the detection of *C. pneumoniae* DNA in CSF. Due to the low *C. pneumoniae* copies that existed in the majority of CSF specimens from MS patients, the TaqMan system may not be sensitive enough for diagnostic purposes, but may be useful as a marker in prospectively following neurological diseases such as MS.

P1378 Use of near patient testing kits for microbiological and epidemiological characterization of group A streptococci

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Objectives: The purpose of this study was to evaluate near patient testing kits (NPTs) for the rapid detection and molecular characterization of group A streptococci (GAS directly from throat swabs, in particular, to determine their use for surveillance of type distributions and macrolide resistance.
Methods: Six commercially available NPTs were evaluated for their sensitivity, specificity and efficacy in the recovery of chromosomal DNA directly from simulated throat cultures. PCR amplification of *emm* genes was undertaken directly from the kit components and detection of *emm* types was determined by a PCR ELISA and/or sequencing.
Results: Recovery of GAS specific DNA was possible with some of the kits and the most user friendly kit (ICON Fx Strep kit) had a detection limit of 10E5 organisms/mL. DNA was extracted more efficiently from the primary lysate of the swab material after exposure to the NPT kit system(s) and was used in the PCR emm capture ELISA. The emm typing data correlated with the results obtained by conventional methods.
Conclusions: The ICON Fx Strep Kit was the most effective and sensitive kit for recovery of DNA. Typing results were available within 24 h of throat sampling and the presence of normal throat flora did not effect the result. This is a novel approach to typing that could also be utilised for detection of a repertoire of virulence genes, in particular those used for antimicrobial resistance.

P1379 Detection of *Chlamydia pneumoniae* in respiratory specimens using real-time NASBA

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Introduction: *Chlamydia pneumoniae* is an etiologic agent of respiratory tract infections in humans. In the past, diagnosis of infection by this organism was based on serology and culture. Real-time nucleic acid sequence-based amplification (NASBA) might offer an alternative.
Objectives: The aim of the study was to develop a real-time NASBA assay for the detection of *C. pneumoniae* in respiratory specimens based on NASBA amplification of a 16S rRNA target sequence using the NucliSens Basic Kit® (bioMérieux).
Methods: Oligonucleotide primers were derived from the *C. pneumoniae* 16S rRNA. The assay was developed using the NucliSens Basic Kit, that includes standardised reagents for nucleic acid extraction, RNA amplification and electrochemiluminescence (ECL) detection. For real-time detection, a molecular beacon was used. Specificity was established on a panel of bacterial strains. The analytical sensitivity of the assays was determined by testing dilutions of a *C. pneumoniae* reference strain. Serial dilutions of *C. pneumoniae* were added to pools of respiratory specimens and treated with protease before

extraction. Subsequently, a number of *C. pneumoniae* positive and negative clinical specimens were analyzed. The results obtained with the real-time *C. pneumoniae* 16S rRNA assay were compared with those obtained by conventional end-point detection using ECL.
Results: Specific detection of the 16S rRNA-derived amplicons was achieved. The real time NASBA assay enabled detection of as little as 0.1 IFU of *C. pneumoniae.* In spiked respiratory specimens, the sensitivity of the *C. pneumoniae* NASBA assay varied between 0.1 and 10 IFU/100 μL sample depending on the nature of the matrix. There was no difference in sensitivity between both detection methods. Finally, 4 PCR positive specimens were positive by both conventional and real time NASBA. None of 100 PCR *C. pneumoniae* negative specimens was positive by both assays.
Conclusions: The NucliSens Basic Kit® combined with real-time detection proved to be a fast, useful and user-friendly diagnostic tool for a NASBA-based assay for the development of a NASBA-based assay for the detection of *C. pneumoniae* in respiratory specimens.

P1380 Serotyping pneumococci by Multiplex PCR

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Objectives: To develop a multiplex PCR-based method for the detection of epidemiologically important pneumococcal serotypes without using immunological techniques.
Methods: Three classes of primers were designed to target capsular polysaccharide (*cps*) genes: (i) the internal control (included in all reactions) (ii) the type-specific, for the detection of serotypes 1, 3, 4, 6B, 14, 18C, 19F, 19A, and 23F; and (iii) the common primers, targeted at conserved *cps* genes. The primers were assembled into two types of multiplex reactions: (i) the group-reaction (GR) with primers common to several serotypes, and (ii) six specific-reactions (SR) with the type-specific primers. Pneumococci were initially screened by the GR that groups sets of serotypes based on common genes. To distinguish between related serotypes within each set and according to the profile obtained in the GR, one of the SR was done. A simple procedure was used for template preparation – a colony was picked with a sterile tip followed by immersion in the multiplex reaction mix. The analysis of the amplification products was done by agarose gel electrophoresis and visual inspection of the patterns obtained.
Results: Out of 446 pneumococci isolated from the nasopharynx of asymptomatic children all were correctly typed by PCR. A total of 294 were identified to the type-level and the remaining 152 presented GR patterns in agreement with the serotype determined by the capsular reaction test. Primers designed to detect serotypes 1, 3, 4, 14, 19F, 19A, and 23F proved to be type-specific, whereas those designed to detect serotypes 6B and 18C were serogroup-specific.
Conclusions: Multiplex PCR showed to be efficient in the detection of the most frequent serotypes colonizing children, while reducing the number of strains that have to be serotyped by conventional immunological techniques. Moreover, it was simple, expeditious (96 samples/day/technician), cost effective and reliable (no specialized expertise is needed) when compared with the capsular reaction test, making it suitable for the analysis of large number of isolates. Preliminary results also show that this method can be used to analyze heterogeneous bacterial samples.

Fungal infections: diagnostics

P1381 PCR-detection of *Candidia albicans* in blood using a new primer pair to diagnose systemic candidiasis

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The opportunistic pathogen *C. albicans* is able to cause disseminated infections in immunocompromised patients. Microbiological methods for the diagnosis of invasive candidiasis have many problem including low sensitivity, requirement to invasive clinical samples such as biopsies or multiple blood cultures and need to expertised laboratory stuff. Since PCR has proven to be a powerful tool in the early diagnosis of several infectious diseases, we applied this approach to a rapid and sensitive detection of *C. albicans* cell in blood samples, for establishment a clinically useful method in diagnosing systemic candidiasis. DNA were extracted from blood samples seeded by serially diluted *C. albicans* cells, by lysing WBC and RBC followed by enzymatic breaking of fungal cell wall and phenol – chlorophorm extraction and alcohol precipitation of DNA, A new primer pair was designed to PCR-amplification of a part of ribosomal RNA gene. The primer set was able to amplify all medically important *Candida* species. When PCR was performed for purified DNA, the sensitivity of the method was 1 pg fungal DNA, whereas the sensitivity for detection of *C. albicans* blastospores inoculated in blood was as few as 10 cells per 0.1 mL of blood. This method could be sensitive and useful for early and rapid diagnosis of systemic *Candida* infections and to simultaneous detection and speciation of *Candida* species by PCR-RFLP method.

P1382 Rapid detection of pathogenic fungi from clinical specimens using real time PCR

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Objectives: Fungal infections are a major cause of infection-related mortality in immunocompromised patients and require early initiation of therapy for successful treatment. However, conventional diagnosis of fungal infections lacks sensitivity and is time-consuming. Since PCR technology holds great promise for the early identification of medically important pathogens, real time PCR was used for diagnosis of fungal infections in clinical tissue samples.
Methods and results: Seven specimens were investigated from six patients with suspected or proven invasive fungal infections. All samples were positive in a panfungal PCR assay. In four samples, *Aspergillus fumigatus* was detected both by a species-specific hybridization assay as well as by sequencing of amplification products. In addition, panfungal PCR detected and subsequent sequencing identified *Candida albicans* in a culture-negative liverbiopsy, *Histoplasma capsulatum* in a bone marrow sample, and *Conidiobulus coronatus* in a facial soft tissue specimen.
Conclusion: Real time PCR appears promising as a means to diagnose invasive fungal infections in human tissue samples. It offers rapid analysis, high specificity and increased sensitivity.

P1383 Comparison of *E*-test method with the proposed procedure for antifungal susceptibility testing of the European Committee on Antibiotic Susceptibility Testing

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Objectives: We have analyzed the correlation between the procedure for antifungal susceptibility testing (AFST) of the European Committee on Antibiotic Susceptibility Testing (EUCAST) and the commercial method *E*-test, a agar diffusion-based procedure for in vitro testing of amphotericin B (AB), fluconazole (FLZ), itraconazole (ITZ), ketoconazole (KTZ), voriconazole (VZ) and flucytosine (FC). The EUCAST has developed a proposed standard for AFST of yeasts based on reference procedure of NCCLS M27-A, but incorporating modifications (RPMI-2% glucose, inoculum size of 105 CFU/mL, spectrophotometrical reading) in order to get an automated AFST and to shorten from 48 to 24 h the incubation period for MIC determination.
Methods: A total of 60 *Candida* spp. clinical isolates (10 each *C. albicans* (CA), C. tropicalis (CT), *C. parapsilosis* (CP), *C. glabrata* (CG), *C. krusei* (CK), *C. lusitaniae* (CL)) were tested. CA ATCC90028, CK ATCC6258 and CP

ATCC22019 were included as QC strains. Triplicate testing on three separate days was performed.
Analysis: (i) Correlation: intraclass correlation coefficient (ICC), over a maximum value of 1.
Results: Overall, the correlation was high with ICC of 0.89 ($P < 0.01$). The lowest ICC values were obtained for CL isolates which exhibited a correlation of 0.7, value with statistical significance. By antifungal agents, the lowest correlation values were obtained for AB, with ICC of 0.73 ($P < 0.01$). The MICs obtained by *E*-test method for azole resistant isolates were as follow: FLZ: >32 mg/L, ITZ: >0.75 mg/L, and VZ: >0.12 mg/L.
Conclusions: (i) The correlation between EUCAST procedure and *E*-test method is high with average correlation values of 0.89. (ii) The method are comparable for all *Candida* species and antifungal agents. (iii) *E*-test method detects azole resistant strains.

P1384 Evaluation of media to enhance conidial production of dermatophytes

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Objectives: Scattered reports about clinical and microbiologic resistance among dermatophytes to antifungals have pointed out need for a reference method for testing the antifungal susceptibility of dermatophytes. Selection of appropriate medium for conidial formation is essential to inoculum preparation for susceptibility testing because dermatophytes are known to demonstrate poor conidial production. The aim of the study was to compare five different agar media by their ability to support conidial formation.
Methods: A total of 33 *Trichophyton rubrum*, 15 *T. mentagrophytes* and eight *T. tonsurans* strains were tested. The five media evaluated were Poor Base Medium (developed by Aho, 1980), Difco oatmeal agar, in-house oatmeal agar, in-house rice-flour agar and Difco rice extract. Dermatophytes were cultivated at +30°C for up to 14 days. The production of conidia was calculated on days 3, 5, 8, 14 using cello-tape preparation. For each isolate the number of conidia was counted in five viewing fields (1000×).
Results: In-house rice-flour agar showed significantly better conidial production on day 8 and on day 14 for all species tested than other media (medians 300; $P < 0.01$). A total of 93% of *T. mentagrophytes*, 88% of *T. tonsurans* and 70% of *T. rubrum* isolates gave conidial production >100 conidia per field on this medium on day 8. The other media supported less than 50% *T. rubrum* isolates to form >100 conidia per field on day 8. More than 85% of *T. mentagrophytes* isolates produced >100 conidia per field on in-house oatmeal and on Poor Base Medium (day 8). The Poor Base Medium gave conidial production >100 conidia per field on day 5 for 86% of *T. mentagrophytes* strains. The conidial formation of 57% and less than 50% of *T. mentagrophytes* isolates was supported by Difco rice extract agar and Difco oatmeal agar, respectively. More than 85% of *T. tonsurans* isolates produced >100 conidia per field on in-house oatmeal and on Difco oatmeal agar. Less than 50% of the conidial growth (>100 conidia per field) of *T. tonsurans* isolates was supported on Poor Base Medium and Difco rice extract agar.
Conclusion: Home-made rice-flour agar was identified as suitable medium to enhance the production of conidia of *T. rubrum*, *T. mentagrophytes* and *T. tonsurans* to ensure appropriate inoculum concentration for susceptibility testing. We suggest the Poor Base Medium for *T. mentagrophytes* conidial formation with minimum time.

P1385 Improvement and limitations of conventional mycologic diagnosis in superficial mycoses

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Background: Low rates of positive cultures from skin and especially the nail samples often impede the mycological diagnosis in superficial fungal infection. No generally accepted guideline exists for interpretation of nondermatophyte positive nail cultures.
Objectives: To analyze results of use of conventional methods for laboratory diagnosis in superficial fungal infections. To find their sensitivity and specificity, and causes of their changes.
Methods: For 5-year-period 1997–2001, 21256 samples from 17 757 patients were processed in mycology unit of Central clinical hospital under Presidential medical center. Nail samples consisted 68%. A total of 3072 positive cultures (14.4%) was obtained, 4210 (19.8%) positive microscopy and 1486 (7%) culture-only positive samples. Performing culture was mandatory for all suspected cases of fungal infections inside that healthcare system.
Results: Sensitivity of microscopy and/or culture was drawn from relation of its positive values to all positive values obtained by either method. The assumption was based on a fact that positive result of either method alone confirms diagnosis of onychomycosis. According to these design, mean sensitivity of microscopy was found as 87.8% and culture as 50%. An increase in culture sensitivity was observed through 5 years. Analyzing the causes of sensitivity improvement, we outline two measures: (i) better sampling by dermatologists, with respect to clinical presentation of onychomycosis, and (ii) paired cultures on a medium with and without cyclohexemide. Total sensitivity increase since 1994 approximated 2.5 times. For more strict distinction between pathogens and contaminants/saprophytes in onychomycosis, we have separately assessed cultures, obtained with concomitant positive microscopy. We have found that with fingernail cultures, proportion of dermatophytes increased from 38.5% to 56.4%, with decrease of *Candida* spp. and molds. Toenail cultures showed similar tendency, with dermatophyte share increased to 86.9%.
Conclusions: Although this fact may reflect the nondermatophyte contamination of the samples, more studies may be needed to differentiate between true and false/mixed nondermatophyte infections. Direct PCR probes for prevalent dermatophytes may help to establish the true rates of yeast and mold nail infections.

P1386 Screening for circulating galactomannan as a marker for invasive aspergillosis in patients with hematologic disease

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Objectives: The sensitivity of a sandwich enzyme-linked immunosorbent assay (ELISA, Platelia Aspergillus kit, BioRad, France) for the detection of *Aspergillus galactomannan* was evaluated in a one year prospective study in a hematological ward with previously low incidence of invasive aspergillosis.
Methods: Eighty-six patients with acute leukemia, aplastic anemia, and patients receiving autologous BMT were tested until the granulocyte count was stable and above 1.0×10^{-9} cells/L or until the patient no longer had signs of clinical infection. Patients receiving allogeneic BMT were tested for a minimum of 8 weeks, patients receiving steroid treatment for GvHD were tested until cessation of steroid treatment and clinical stability. 680 plasma specimens were collected from patients in the study biweekly for investigation of galactomannan levels.
Results: During the testing period no patients died of proven aspergillosis, one patient died of probable aspergillosis. This patient tested galactomannan positive (index value 5.816) 4 days prior to death. One day after the positive galactomannan testing, *A. fumigatus* grew from sputum, tracheal and bronchial secretions. Two patients tested falsely positive (index value 1.973 and 1.606), one of these died of autopsy-verified invasive *Candida* 3 days after positive galactomannan testing. None of the galactomannan positive patients showed positive gray-zone values at any point during the testing. Thirteen patients showed positive gray sone values (index value = 0.8–1.5) without any signs of infection. 640 samples tested negative.
Conclusion: Due to the low number of patients testing galactomannan positive and to the lack of gray zone values in this single patient, and to the high number of falsely positive gray zone values, the galactomannan test was not introduced as standard test for the hematological patients in our hospital.

Diagnostic methods in parasitology

P1387 IgG avidity test for acute toxoplasmosis in pregnant women in County of Istria

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Objectives: It is of great importance to confirm particular antibody classes using a combination of suitable serological tests for exclusion of recent primary infection with toxoplasma in pregnant women. Toxoplasmosis testing should be done in early pregnancy, using ELISA or IFA to detect specific classes of IgM and IgG antibodies. When necessary further IgA antibody and Western blotting testing can be applied. Determination of precise date of seroconversion is often difficult because of persistence of IgM and high IgG titter. This leads to difficulties in distinguishing reactivation, reinfection, polyclonal stimulation, presence of residual or nonspecific IgM, and subsequently leads to unnecessary treatments, amniocentesis and even abortion.
Methods: *Toxoplasma gondii* imuno-globulin G (IgG) avidity enzyme-linked fluorescent assay is based on measurement of avidity (binding strength)-specific IgG antibody to antigen in presence of urea buffer. Characteristic for early phase of infection is dissociation of low-avidity IgG antibodies from antigen. Representative of prior infection is increase of IgG antibody and antigen binding affinity (high avidity), and the absence of dissociation in urea buffer. Avidity assay results are generally expressed as an avidity index (AI). In combination with other serological tests enables rapid timing of primary infection or differentiation of primary or secondary (persistent or reactivated) infection. From a resident population of 200.000, during one year 960 sera samples from 937 pregnant women were analyzed for presence of *Toxoplasma gondii*-specific IgM and -IgG antibodies.
Results: IFA and ELISA test were performed and 32% of samples were positive for presence of *T. gondii* IgG antibodies (hence remaining 68% are susceptible for primary infection during pregnancy). In seven pregnant women (0.74%) IgM and IgG antibodies both were identified. IgG avidity test indicated high avidity in four women, thereby excluding the acute primary infection. In the other three with low IgG avidity artificial abortion was performed in one, and spiramycin therapy initiated in remaining two pregnancies with diagnosed acute infection (All assays BioMérieux, France).
Conclusion: *T. gondii* IgG avidity test enables acute infection exclusion in IgM positive pregnant women and potentially reducing unnecessary treatments by at least 50%.

P1388 Toxoplasmosis in Kuwait: improved diagnosis based on quantitative immuno-assay

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Introduction: *Toxoplasma gondii*, is an opportunistic intracellular coccidian pathogen that causes a benign infection in immunocompetent individuals but has emerged as a major opportunistic pathogen immunocompromised patients. The main objective of this study was to determine the seroprevalence rate among healthy adults and pregnant women in Kuwait by a rapid and specific diagnostic assay to detect *Toxoplasma*-specific IgG antibodies.
Study Groups and methods: (1) *Control groups:*
(1.1) Positive for *Toxoplasma* IgG antibodies: 50.
(1.2) Negative for *Toxoplasma* IgG antibodies: 50.
(1.3) Randomly selected negative sera: 150.
(2) Adult Healthy Individuals: 1475. All major ethnic and foreign national groups living in Kuwait were included.
(3) Pregnant women: 225 (75 each in 1st, 2nd and 3rd trimester).
Screening Assays for Specific Toxoplasma antibodies: The following screening assaying were used: I. VIDAS IgG/IgM (bioMerieux Vitek, MO, USA). II. Mercia Toxo Enzyme Immunoassay (Syva, Palo Alto, USA). III. Toxolatex Immunoassay (Fumouze Labs, Le Malesherbes, France). All equivovcal results were confirmed by indirect fluorescent antibody assay (Virgo, Pharmacia Diagnostics, NJ, USA).
Results: The performance of the three immunologic assays to detect antibodies to *T. gondii* was determined by screening the control group. VIDAS detected 53 positive cases and Mercia EIA detected 51 cases, and 4 cases were equivocal. VIDAS assay had the highest sensitivity (9603%) and specificity (99.5%) of the three assays. The seroprevalence rate of antibodies to toxoplasma parasites in our study population ranged from 25% in Philippinos to 69.3% in Arab nomads. 42% of the urban Kuwaiti population showed significant level of antibodies to *T. gondii*. 45.7% of the pregnant women had significant levels of *Toxoplasma* IgG antibodies. Incidence during different trimesters was approximately the same, i.e. 30%. Further, comparative data analysis is also presented with other studies conducted in the Gulf region.
Conclusion:
1. VIDAS immunoassay had the highest sensitivity (96.3%) and specificity (99.5%).
2. The seroprevalence rate of antibodies to toxoplasma parasites in various study populations ranged from 25% to 69.3%.
3. 42% of the urban Kuwaiti population showed significant level of antibodies to *T. gondii* that is higher than that in Saudi Arabia and UAE (31%).
4. The seroprevalence rate in pregnant women was 45.7%.

P1389 Evaluation of two enzyme-linked immunosorbent assays and one immunochromatographic rapid assay for the detection of *Giardia lamblia*-specific antigens in fecal specimens of patients with giardiasis

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Objectives: The estimation of sensitivity and specificity of two enzyme immunoassays (Alexon-Trend ProSpecT Giardia Microplate Assay and Cypress Diagnostics Giardiasis Ag ELISA) and one immunochromatographic assay (Becton Dickinson ColorPAC Giardia/Cryptosporidium Rapid Assay) in comparison with microscopic detection of *Giardia* cysts and trophozoites in patient fecal specimens, in order to evaluate their usefulness as alternative methods for diagnosing giardiasis.
Methods: During the last 2.5 years, 5800 fecal specimens were examined by the conventional microscopic examination (direct wet preparation and after concentration) for ova and parasites. All the positive fecal specimens for cysts and/or trophozoites of Giardia using the ova and parasite examination were stored fresh (unpreserved) at -70°C. These fecal specimens were retrospectively examined for the presence of *Giardia*-specific antigens with the three methods mentioned above using the manufacturer's recommended procedure. Also 52 fecal specimens negative for *Giardia* by ova and parasite examination were simultaneously tested.
Results: Sixty-one fecal specimens (29 from Greek people and 32 from immigrants) of the 5800 examined were found positive for *Giardia* by microscopic examination (1.05%). *Giardia*-specific antigen 65 (GSA 65) was detected in all the 61 positive fecal specimens tested by Alexon ProSpecT Giardia Microplate Assay (sensitivity 100%). Two fecal specimens of the 61 (with the lowest absorptions in the Alexon-Trend assay) were found negative tested by the immunochromatographic assay (sensitivity 96.72%), and one of them by the Cypress diagnostic kit as well (sensitivity 98.36%). The 52 negative samples were found negative in all diagnostic kits (specificity 100%).
Conclusions: (i) ProSpecT Giardia Microplate Assay, which is as sensitive and specific as the microscopic detection of the parasite and secondly Giardiasis Ag ELISA (Cypress Diagnostics) could offer alternative and complementary methods to routine ova and parasite examination, especially in chronic diarrhea or clinical suspicion of giardiasis, when the first microscopic examination of the stool is negative. (ii) The ColorPAC Giardia/Cryptosporidium Rapid Assay could be applied as a rapid screening test, though a negative result does not firmly exclude the infection.

P1390 Utilization of flow cytometry in diagnosis of microfilaria infection

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Introduction: Some authors have recently proposed automated light depolarization analysis of whole blood as a useful tool for diagnosing malaria

infection. During our study we observed abnormal patterns in samples from patients with other parasite infections.
Aim of study: We try to establish a relation between these patterns and microfilaria infection during ordinary hematologic study of samples.
Material and methods: Tests were made on 401 peripheral blood samples collected in tubes containing EDTA using the Cell-Dyn 4000 analyzer (ABBOTT-diagnostics). The analytical principles are the same as those upon which flow citometry is based. The leukocyte differentiation is obtained by measuring the amount of laser light dispersed by a cell at different angles. Microfilaria infection was diagnosed by 'Knott Concentration Method' followed by parasite visualization under light microscope.
Results: A total of 22 samples were proved positive for infection by microfilaria. Nineteen out of them corresponded to *M. perstans*, two to Loa–loa and one mixed infection. Observing the graphs from flow citometry of these samples, the main abnormal patterns were found when green-coded events fell outside and parallel to the normal eosinophil 'footprint' (also green). Other abnormal events were also present in the 'size vs. granularity' graph. These abnormal populations could represent microfilaria fragments able to disperse and depolarize laser light.
Conclusion: We suggest the utilization of flow citometry as a useful complementary tool in diagnosis of microfilaria infection, specially in those nonendemic areas where there is little awareness of these infections and could be misdiagnosed.

P1391 Examination of feces for the detection of parasites: a comparison of two methods

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Background: The diagnosis of intestinal parasitic infection is confirmed by the recovery of helminth larvae and eggs, protozoan Trophozoites and cysts.
Objective: A comparison was made between two methods for the detection of protozoa cysts in human fecal sediments of formalin–ether and formalin–gasoline technique.
Material and methods: A total of 400 fecal samples were examined by two methods, in order to compare the value of two techniques. 342 different parasites were identified by both techniques. Forty-four parasites were found only by formalin–ether technique and 15 parasites were found only by the formalin–gasoline method.
Result: The sensitivity, false negative and negative predictive value of these techniques were determined.
Conclusions: The results of this study show that formalin–gasoline method can be as sensitive as formalin–ether technique. However, it can be, less dangerous, and cost benefit than formalin–ether technique.

P1392 Evaluation of new commercialized systems for the concentration and staining of intestinal parasites in fecal specimens under routine laboratory conditions

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Objectives: Because, intestinal parasites are intermittent shaded and vegetative stages of protozoa are fragile, the routine diagnosis of enteric parasites infection is complex and confusing. The development of the new commercialized systems for the concentration and staining of parasite could resolve many problems met in the diagnosis of intestinal parasites. The purpose of this study is to compare the recovery and identification of intestinal parasites in fecal specimens, using new commercialized systems for the concentration (Parasep 145600 and SpinCon) and staining (EcoStain), vs. our routine methods consisting in Triple-Feces test (TFT) recently developed in Holland.
Methods: Fecal specimen collected from 250 patients suspected to have parasitologic diarrhea were examined according to our specific TFT protocol. TFT consists in three samples collected on 3 consecutive days (two with SAF preservative and one fresh specimen) which are examined with and without concentration techniques and a permanent staining (Chlorazol Black). Results obtained with the two commercialized concentration systems (Parasep 145600 and SpinCon) were compared with our conventional ether–sedimentation procedure. Permanent stained smears systematically prepared from the stool specimens were assessed for morphology, clarity of nuclear and cytoplasmic detail.
Results: Over the 250 patients examined, 37% were found positive for potentially pathogenic parasites after the examination of the complete TFT test, whereas the technical performance using one of the 3 concentration procedures were comparable ($P > 0.05$) and allow us to recover parasites in only 11% of patients. Moreover, no significant differences were found in the numbers and morphology of organisms seen in the concentration sediment regardless the technique used. Few clinical relevant differences was seen between EcoStain and Chlorazol Black staining. The same organisms were usually identified in both staining, with the exception of situations in which organism numbers were characterized as rare.
Conclusion: The use of TFT protocol allowed us to increase the recovery of intestinal parasite when we compared with the concentration method only. Our study suggests that commercialized concentration (Parasep and SpinCon) and permanent staining (EcoStain) systems are reliable and easy to use for diagnosis of intestinal parasites in routine clinical practice.

P1393 A quality control survey on malaria microscopy in State and private laboratories in Ardabil province: Iranian border with Republic of Azerbaijan

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Background & objectives: Quality control of microscopic diagnosis has an important role in evaluating the Malaria control programs. We studied the quality of simple microscopic diagnosis of Malaria in Ardabil province north-western Iran.
Methods: We re-examined 389 blood smears examined for malaria by clinical laboratories of Ardabil province in 1999. All positive cases and a threefold number of negative reported smears were randomly selected to be reexamined by expert microscopists in parasitology laboratory of public health faculty, Tehran University. The results were considered as standard test and sensitivity and specificity of microscopic diagnosis in personal laboratories and special malaria state laboratories of Ardabil province were calculated.
Results: From 389 blood smears reexamined during survey 119 found to be positive and 270 negative for malaria. 20 (16.8%) out of 119 cases of malaria were not diagnosed and were reported negative by local laboratories. sensitivity and specificity was calculated to be 87.3 and 98.5, respectively, for local diagnosis. Sensitivity of tests was 87.3 and 78.5% for personal and state laboratories, respectively. Specificity was 97.9 and 100%, respectively, for personal and state laboratories. We noticed that personal laboratories did not examine thick blood film and state laboratories did not examine thin blood film. We were obliged to reexamine the smears as was examined by source laboratories. We followed up retrospectively the people who with positive smear for malaria that local laboratories had not diagnosed their disease and found that most of them had later been diagnosed for malaria during the next coming year after the date of false negative report for smears. But some lived without any later diagnosis of malaria.
Conclusion: Although the personnel in state laboratories had a long time experience in malaria microscopy, Sensitivity of microscopic diagnosis of malaria was very low and unacceptable. Considering the exclusive use of thin blood films in personal laboratories, the real sensitivity of microscopic diagnosis of malaria is estimated to be much lower than the value calculated for this sector, which could not be acceptable either.

Neonatal infections and pediatric intensive care

P1394 A 5-year retrospective study of yeast and fungi isolated from PICU patients and the role of antifungal prophylaxis

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Objectives: Patients in pediatric intensive care units (PICU) are a known to be at a high of acquiring *Candida* infection with antibiotic use, catheterization and length of stay being recognized as risk factors. In order to reduce the incidence of systemic *Candida* infection targeted prophylaxis with fluconazole was introduced at the end of 1999. This study was undertaken to examine the effectiveness of targeted fluconazole prophylaxis in PICU patients.
Methods: Patients who were prescribed more than two antibiotics, colonized with *Candida* spp. at more than one site or who had been in PICU for more than 2 weeks and were thought to be at risk of developing a fungal infection, were given a prophylactic dose of fluconazole, 2–4 mg/kg/day. Blood and surveillance cultures were taken on clinical grounds and there was no change in sampling regimens after the introduction of fluconazole prophylaxis. Data for the study was obtained retrospectively from the microbiology department computer system.
Results: A total of 1230 isolates of *Candida* spp. and other fungi were isolated from 335 PICU patients during the five year period 1997–2001. The most common species isolated was *C. albicans* ($n = 1014$; 82%). Of the nonalbicans *Candida* species isolated the next most common were *C. parapsilosis* ($n = 97$; 8%) and *C. glabrata* ($n = 47$; 4%). The remaining 6% of non – albicans *Candida* species were made up of *C. lusitaniae*, *C. krusei*, C. topicalis, unidentified yeasts and filamentous fungi. In 1997–1999, 20, 5 and 13%, respectively, of all blood culture isolates were due to *Candida* spp. or other fungi. In 2000 and 2001 there were no *Candida* spp. or other fungi isolated in blood cultures on the PICU.
Conclusions: Our results, however, have shown that the use of fluconazole prophylaxis significantly reduces the risk of bloodstream infection with *Candida* spp. In addition, there has not been a significant increase in non albicans *Candida* infection or colonization.

P1395 Sepsis in the neonatal surgery unit

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Objectives: Bacterial infection in neonatal surgical units remains a major problem. Neonatal sepsis can arise from a variety of causes, one of which may be bacterial translocation, that is the passage of bacteria from the gastrointestinal tract to extra-intestinal sites, typically across the lamina propria to the mesenteric lymph nodes and hence to the blood. It is possible, in the neonatal setting, that overgrowth of bacteria in the proximal gut is associated with increased bacterial translocation and hence sepsis. We aimed to determine firstly which organisms are the major causes of sepsis in a neonatal surgical unit and secondly whether there was an association between bacterial colonization of the proximal bowel and the subsequent development of sepsis.
Methods: A retrospective analysis of blood cultures and gastric aspirates was undertaken for all cultures received from the neonatal surgical unit between 1999 and 2002. The time of gastric aspirate sampling in relation to a positive blood culture and the bacteria found in the gastric aspirates were recorded. Standard methods for culture and identification of bacteria were used.
Results: Overall, 1258 blood cultures were collected from 291 patients and 1464 gastric aspirates from 264 patients. The commonest organisms isolated from blood culture were coagulase negative staphylococci (41%) and various aerobic gram negative rods (53%). 68% of patients with positive blood culture had a positive gastric aspirate, compared with 48% of those with negative blood cultures. Of those patients with a positive blood culture 17% had the same organism in both gastric aspirate and blood culture.
Conclusion: In certain patients, positive gastric aspirate culture predicts subsequent bacteremia. In addition, certain organisms are more frequently found synchronously in gastric aspirates and blood cultures. Further, work is needed to relate bacteriological findings with clinical conditions to increase the predictive power of surveillance cultures.

P1396 Nosocomial bloodstream infections in a national pediatric burn center in Hungary

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Objectives: The purpose of this study was to determine the microbial pathogens causing nosocomial bloodstream infections in pediatric burn patients.
Methods: A retrospective survey of medical records was performed. Children treated and consecutively hospitalized in the burn center between 1 November 2001 and 30 November 2002 who had nosocomial bloodstream infections were identified. A review of causative microbial agents, age, gender and percentage of total body surface area burned was done.
Results: Over a 13-month period from a total of 59 burn patients seven (11.8%) had one or more episodes of nosocomial bloodstream infections. All seven children (five males, two females), ages 9–103 months, had experienced thermal injury covering at least 32% of their body surface (range = 32–75%, mean = 52.7%). The most usual bacterial pathogen was *Pseudomonas aeruginosa* followed by *Staphylococcus aureus*, coagulase-negative Staphylococcus and *Klebsiella pneumoniae*. Two persons had fungal infections. Two of the 59 patients died, both of *Pseudomonas aeruginosa* sepsis.
Conclusions: *Pseudomonas aeruginosa* was the leading cause of nosocomial bloodstream infections and the only cause of mortality in pediatric burn patients in one center over a 13-month period.

P1397 Early- and late-onset sepsis at neonates. Clinical and microbiological characteristics

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Objective of the study: To determine the rate of early and late-onset sepsis of newborns treated in the NICU of Department of Paediatrics, Semmelweis University, Budapest, Hungary.
Patients and methods: 1008 newborns were transmitted to our unit in a tree years long period of time (2000–2002). 719 of their required long-term respiratory therapy. The number of newborns with very low birth weight (<1500 g) was 220. All newborns had a sepsis screen immediately or later if the clinical signs indicated it.
Results: 108 newborns had such a sepsis which was evidenced by positive blood culture, 44 of 220 newborns with very low birth weight (Group I.) and 64 of 788 other prematures and term babies (Group II.). The incidence of early onst sepsis in the Group I. was 3/44 (7%), and this was in the Group II. 24/64 (38%). The distribution of the pathogens at early onset sepsis was the following: Gram-positive organisms 87.5%, Gram-negative organisms 12.5%. This at late-onset sepsis: Gram-positive organisms 63%, Gram-negative organisms 44% and fungi 8.6%. Mortality: 1/27 at newborns with early onset sepsis and 10/81 (12%) at newborns with late-onset sepsis.
Conclusions:
1. The rate of early onset sepsis at newborns with very low birth weight is rare.
2. The most frequent pathogens at early onset sepsis are Gram-positive organisms.
3. Prematures with very low birth weight has a high risk for late-onset sepsis.
4. The incidence of Gram-negative organisms at late-onset sepsis is high.
5. The late-onset sepsis is connected with higher mortality rate as the early onset one.

P1398 Evolution of group B streptococcal early neonatal sepsis rates during 6 years in a university hospital (Madrid, Spain)

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Objective: The aim of this study was to know the rates of group B streptococcal early neonatal sepsis (SGBENS) in our hospital from 1995 to

2000, and to evaluate the impact of the implementation of the CDC's guidelines (1995–1997) and after Society of Clinical Microbiology of Madrid's (SMMC) guidelines.
Methods: We study all the neonates in our hospital between 1995 and 2000: 1995, 2439; 1996, 2283; 1997, 2236; 1998, 2228; 1999, 2819; and 2000, 3039. During 1995–1996, the universal prenatal screening for detection vaginal and rectal group B Streptococcus (GBS) colonization in our area (area 2), between 24 and 28 weeks' gestation, began. Ampicillin was used when the intrapartun antibiotic prophylaxis was necessary (2 g at least 4 h before the labor, and 1 g/4 h). In 1998, we started to attend the labors of area 1, where there was no universal prenatal screening for GBS. In 1999, they began the universal prenatal screening for GBS (SMMC's guidelines: swabbing both the lower vagina and rectum were inoculated on a selective agar plate and also were immersed in Todd-Hewitt. Early neonatal sepsis was considered when the neonate had GBS bacteriemia and signs and or symptoms of infection.
Results: The GBS colonization rates were: 1996, 15%; 1997, 20.8%; 1998, 19.6%; 1999, 18.8%; and 2000, 20%. The number of culture that were positive for GBS is: 1995, 6‰; 1996, 2‰; 1997, 1‰; 1998, 7‰; 1999, 5‰; and 2000, 4‰ live births. The evolution of early neonatal sepsis rates were: 1995, 2.4‰; 1996, 0.8‰; 1997, 0.4‰; 1998, 3.14‰; 1999, 1.7‰ and 2000, 0.9‰ live births.
Conclusions:
1. The interanual rate of GBSENS in our hospital is 1.66‰ live births.
2. In 1995 coinciding with the beginning in our area (area 2) of the implementation of the CDC's guidelines, the incidence of early onset disease declined: (2.4 in 1995 to 0.4‰ live births in 1997).
3. The start in our hospital of the labors from area 1 (without universal screening for GBS) was associated with an increase of the GBSENS rates (from 0.4 to 3.14‰ live births).
4. Coinciding with active prevention efforts in 1999–2000 in both areas (area 1 and 2), the incidence of early onset disease declined to 0.9‰ live births in our hospital.

P1399 Coagulase negative Staphylococci with reduced susceptibility to teicoplanin in bloodstream infections of neonates in a neonatal intensive care unit

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Objectives: The purpose of this study was to characterize the species distribution of nosocomial CNS isolates, recovered from blood cultures in the NICU and to determine their susceptibility to antimicrobial agents for the emergence of reduced susceptibility to glycopeptides.
Material: Methods: A total of 6.238 blood cultures were screened for aerobic, anaerobic microorganism and fungi for a 2 years period (2000–2002). Species identification was performed with an automated system (PASCO-DIFCO) and confirmed by the Api Staph. System (Bio-Merieux, France). Susceptibility to antibiotics was detected by the diffusion method according to the NCCLS guidelines. Minimal inhibitory concentrations (MICs) were determined by broth microdilution technique (PASCO-DIFCO) and *E*-test method. (AB BIODISK, Solna Sweden). *E*-test was used as the method of choice for detection of CNS strains with reduced susceptibility to glycopeptides.
Results: Bacteremia was diagnosed in 411 cases (6.7%) of the hospitalized neonates. of the 248 isolated Gram positive cocci, 214 (86.2%) proved to be CNS. The species distribution was as follows: *S. epidermidis* 92.5%, *S. warneri* 3.7%, *S. simulans* 2.3%, *S. haemolyticus* 1% and one isolate (0.46%) for the species *S. hominis S. xylosus* and *S. saprophyticus*. All CNS isolates were susceptible to vancomycin. Most of the *S. epidermidis* isolates were resistant to oxacillin (76.1%), macrolides (50.5%), clindamycin (18%), gentamicin (52%), fluoroquinolones (7%) and teicoplanin (2% – MICs > 32–128 μg/mL). Two *S. epidermidis* strains resistant to oxacillin (MICs > 128–256 μg/mL) and teicoplanin (MICs 32–64 μg/mL) showed a borderline susceptibility to vancomycin (MIC 6 μg/mL). Finally an intermediate resistance to teicoplanin (MICs 16–24 μg/mL) was detected in 12 CNS *C. methicillin* resistant strains.
1. *S. epidermidis* strains (92.5%) are the predominant Gram positive causative agents of NICU acquired infections.
2. The emergence of CNS isolates with reduced susceptibility to teicoplanin emphasize the need for glycopeptide resistance surveillance especially in hospitals with high rates of oxacillin resistant staphylococci.

P1400 *Stenotrophomonas maltophilia* in a neonatal intensive care unit

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Objectives: To determine the prevalence, the susceptibility to antimicrobials and genotypic relationship of clinical isolates of *Stenotrophomonas maltophilia* in a neonatal intensive care unit.
Methods: *S. maltophilia* strains were isolated from different samples (blood culture, respiratory tract and urine) of neonates treated in the neonatal intensive care unit between January 2001 and December 2002. The strains were identified using the ATB and Vitek systems (bioMérieux) and genotyped with AP-PCR (ERIC-2). Susceptibility to antibiotics were evaluated by disk diffusion method and by *E*-test strip.
Results: A total of 45 *S. maltophilia* were isolated from colonized or infected 31 neonates, which represented 8% of the total of Gram-negatives recovered over the study period and 30% of the total of nonfermenters. There was no considerable difference in clinical severity between patients infected or colonized with *S. maltophilia* and those of noninfected ones. The risk factors for bloodstream-, and respiratory tract infection or colonization were the low maturity (delivery at an average of 28.8 gestation weeks), the low birth weight (average: 1281.4 g), the prolonged hospital stay, the mechanical ventilation (100%), the different indwelling catheters (100%) and previous broad spectrum antibiotic prophylaxis (carbapenems). The incidence of resistance to aminoglycosides, ceftazidime, ciprofloxacin and moxifloxacin was 100, 34, 76 and 0%. No resistance strain to cotrimoxasole was found. Based on the results of AP-PCR the strains could be divided 10 different clones.
Conclusion: *S. maltophilia* strains seems to be the leading causes of nosocomial infections nowadays, especially in intensive care units. Management of *S. maltophilia* infections may be difficult due to the inherent multidrug resistance of starins. The newer fluoroquinolones could be considered as good option for the treatment of such infections. AP-PCR is a useful tool for evaluating nosocomial infections caused by *S. maltophilia*. The high genetic diversity among the isolates probably means, that the multiple independent environmental sources are more relevant than the cross-transmission in nosocomial infections, therefore the independent infection are more probable.

P1401 Prognostic factors in neonatal infections: experience from 246 neonates at a single neonatal referral center

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Objectives: To assess prognostic and risk factors of neonatal infections in neonates.
Methods: We investigated prognostic factors for inferior outcome in 246 neonates from a National Referral Clinic of Neonatology, hospitalized in years (1999–2000) in Slovakia (5.5 million inhabitants). We detected risk factors, mother's risk factors, and types of isolates, etiology, diagnostic indicators, localization of infection site, therapy and prognosis.
Results: Of 246 patients, 16 had inferior prognosis, which was defined as: death of infections, death of underlying diseases with infections, intraventricular hemorrhage, and periventricular leukomalacia. Risk factors for inferior outcome were: very low body weight (<1000 g) (43.75% vs. 0.43%, $P < 0.0001$), <28 weeks gestation (37.5% vs. 0.87%, $P < 0.0001$), respiratory distress syndrome (25% vs. 3.04%, $P < 0.003$), total parenteral nutrition (31.25% vs. 10.87%, $P < 0.03$), cerclage in mothers (imminent premature delivery or mother with history of abortion) (25% vs. 5.22%, $P < 0.01$). Bacteremia (positive blood culture) was predictor for inferior outcome (31.25% vs. 8.7%, $P < 0.014$) as well as meningitis (12.5% vs. 0.43%, $P < 0.011$). High procalcitonin levels (31.25% vs. 5.22%, $P < 0.002$) were significant predictors of inferior outcome as well as septic score > 2 points (25% vs. 6.52%, $P < 0.025$).
Conclusion: Bad prognostic factors in neonates associated to risk neonate's factors and several neonatal infections, what showed high procalcitonin level and septic score as diagnostic indicators.

P1402 Infections of neonatal age in very low birth weight neonates are not associated with higher attributable mortality

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Methods: We analyzed risk factors and outcome of infections, appearing in very low birth weight (VLBW) neonates to other neonates, within all infectious episodes, appearing in 2 years in tertiary referral neonatal center: 246 cases within 01/01/1999 to 01/01/2001were analyzed. We used a univariate analysis, to assess risk factors for neonatal infections according to the birth weight.
Results: Among 246 cases, 36 patients had very low birth weight <1500 g (VLBW) and 210 patients had birth weight >1500 g (non-VLBW). Risk factors, which included: ventilatory support ($P < 0.01$), total parenteral nutrition ($P < 0.0001$), central vascular catheter ($P < 0.01$), percutaneous arterial catheter ($P < 0.0017$) and respiratory distress syndrome ($P < 0.012$), were significantly more frequently observed in VLBW newborns in comparison to non-VLBW infants. In a univariate analysis of risk factors related to mother, previous cerclage was more frequently observed ($P < 0.0005$) in VLBW newborns. Positive blood cultures and/or catheter tips showed, that the rate of bacteremia/sepsis in <1500 g birth weight newborn infants (41.67% vs. 7.62%, $P < 0.0001$) was higher than in those with >1500 g birth weight. From etiology, Klebsiella/Enterobacter was more frequently in VLBW newborns (69.44% vs. 40.95, $P < 0.0027$). Concerning diagnosis of infection, positive procalcitonin (PCT) test (22.22%, $P < 0.0009$), leukocyte count >30000 ($P < 0.028$), and septic score >2 points ($P < 0.00037$) were more significantly related to VLBW neonates. Mortality on underlying disease was higher in VLBW neonates than non-VLBW (8.33% vs. 0.00%, $P < 0.0029$). Also periventricular leukomalacia (PVL) ($P < 0.02$), and intraventricular hemorrhage (IVH III./IV.) ($P < 0.0099$) were significantly more frequently observed in VLBW neonates.
Conclusion: Attributable (infection related) mortality was similar in both groups. The most important factor, why infection has not contributing to death is strict infection control strategies at the Neonatal intensive care clinic implanted after 1996.

P1403 Hemolytic activity of *Staphylococcus cohnii*

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Objectives: To study hemolytic activity of 297 strains of *S. cohnii* isolated from the environment and skin swabs from personnel and patients – premature infants in the intensive care unit of a pediatric hospital. Staphylococcal hemolysins are important virulence factors of these bacteria. *S. aureus* produces alpha, beta, gamma and delta hemolysin. Coagulase-negative staphylococci synthesise alpha and beta toxins rarely, however, delta-hemolysin in these bacteria was found very often. *S. cohnii* show hemolytic activity.
Methods: Hemolytic activity was checked on TSA plate with sheep, rabbit or human erythrocytes. CAMP test was used to detect delta-hemolysin. CAMP test with *Streptococcus agalactiae* was used to study the production of beta-hemolysin. Alpha-hemolysin production was checked with the use of the antiserum. Hemolytic activity was quantified at 541 nm. Proteins from the culture supernatants of the two strongly hemolytic strains were fractionated by precipitation with ammonium sulfate and separated in native-PAGE. Alpha-hemolysin was identified on nitrocellulose with specific antiserum. Molecular weight of hemolysins was estimated by SDS PAGE and native PAGE.
Results: *S. cohnii* strains used in this study hemolyzed all types of red cells. CAMP test was positive for 92% strains of *S. cohnii* ssp. *cohnii* and 75% of *S. cohnii* ssp. urealyticus. The activity of beta-hemolysin was not found. Inhibition test of hemolysis with rabbit antiserum against alpha-hemolysin and native-PAGE/Western blot proved alpha-hemolysin absence. Hemolytic activity was associated with proteins precipitating between 30 and 60% saturation of ammonium sulphate. These proteins were positive in CAMP test, which suggested the presence of delta-hemolysin. Native-PAGE showed high heterogeneity of hemolysins of both subspecies of *S. cohnii* studied. These hemolysins maintained biological activity after SDS PAGE separation and had low molecular weight. *S. cohnii* hemolysins were found to be stable in high temperatures and could be lyophilized without loss of activity and stored in PBS solution at room temperature for several days.
Conclusion: The results of our preliminary study can be summarized as follows: hemolytic activity of *S. cohnii* ssp. *cohnii* and *S. cohnii* ssp. urealyticus strains is connected with the production of proteins similar to delta-like hemolysin found in other coagulase-negative staphylococci. The study of hemolytic activity of *S. cohnii* will be continued.

P1404 Cell surface characteristics of *Staphylococcus cohnii*

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Objectives: Adhesion is one of the most important stages in the pathogenesis of microbial infection. Numerous multiresistant *Staphylococcus cohnii* isolates were found in the ICU of a pediatric hospital. The strains of this species are characterized by strong hydrophobicity and also the ability to form slime.
Methods: The research has been performed on three strains of *S. cohnii* which showed various ability of slime production, hydrophobicity in SAT and MATH test, and also rough type of growth. The bacterial cells were fractionated to obtain cell surface glycoproteins. The slime of *S. cohnii* was isolated after inoculating a staphylococci over a dialysis membrane of CRA plates. Bacterial cells were treated by a gentle sonication as long as the cluster cells were disengaged and then liofilizate was analyzed. Cell surface proteins were isolated using disintegrator and lysostaphin. The glycoproteins were analyzed by SDS-PAGE.
Results: Two of the investigated isolates originated from the ICU environment (ZMF264 and ZMF325) and one from the skin swab of a hospitalized infant (ZMF138). The two last ones produced slime. Isolates ZMF264 and ZMF138, but not ZMF325, formed conglomerates when cultured in TSB medium and were hydrophobic in the SAT test. However, MATH test for hydrophobicity estimation showed that all strains were hydrophobic. The lowest affinity to hexadecan was presented by ZMF138. All strains showed the ability to be transferred to organic phase in acidic pH. All strains, independently of the origin, slime, and cell surface hydrophobicity, firmly adhered to PCV. SDS-PAGE analysis of extracellular proteins separated on dialysis membrane revealed that strains able to produce slime showed additional thick bands close in weight, but not identical (about 100 kDa) for both producers of slime. The substance responsible for conglomerate forming (SRCF) released from rough strains, separated in SDS-PAGE, formed several bands 14–94 kDa in size. The presence of polysaccharides components in the investigated glycoproteins was confirmed. Small differences in cell wall proteins composition were also observed.
Conclusions: The cell surface of *S. cohnii* is a complex structure, which consists of many glycoproteins. These substances had been implied in the initial adhesion stage. This concerns also, although to a different degree, adherence to surface and clusters formation, which may be stimulated by increase ionic strength of the environment.

P1405 Screening of environmental microorganisms in the premature newborn hospitalization unit and in surgical rooms

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Purpose: The screening and study of the environmental microorganisms in a unit for the hospitalization of premature newborns and in the surgical rooms of a pediatric hospital.
Material and methods: The study was carried out in two time periods, with a gap of two months and concerned: (i) premature unit: samples for cultures were taken at the same time from the interior of incubators, the baby weighing machine, the changing bench and the air conditioners. (ii) Operating room: surgical table, tool table, anesthesiology machine, sterilization bench, changing table, sink, sterilization paddle-steamer. Sampling was carried out with the special nutritional count-tact plates (tryptone-soya agar), the incubation of the plates was done in CO_2 and air, respectively, for 24–48 h and the identification of isolates (biochemical and phenotypic) to species level, with the conventional techniques. It was evaluated the isolation of over 24 colonies of the same microorganism per plate.
Results: (i) Premature unit: From the total number of 18 samples, 15 produced no growth (negative cultures). At the sample taken from the baby weighing machine they were isolated coagulase-negative staphylococci and Gram-negative, oxidase positive bacilli (Pseudomonas). From the layer of the incubator they were isolated fungi and from the changing layer of the newborns they were isolated co (–) staph. and Gram (–) bacteria. (ii) Surgery room: from the total number of 20 samples (at the first screening), 12 of the

samples were negative. Eight of the samples regarded the one surgical table, the anesthesiology machine, the tool table and the sink were positive [they were isolated: co (–) staph, fungi, pseudomonas and pasteurella]. During the second sampling and following the intensification of the cleaning methods of the surgery room, including the changing of the used antiseptics, we had all 20 relevant samplings negative.
Conclusions: There were minimal differences between the amount of growth and the identification of organisms in the premature unit and the surgical room. No correlation was observed between the above-mentioned environmental strains and the potential isolated organisms from cultures (blood or swabs) taken from operated children or premature newborns hospitalized in the unit. We note the beneficial results of the careful cleansing policies and the choice of the appropriate special antiseptics in the area of the surgery room.

P1406 Device-associated infection rate in the neonatal care unit

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Objectives: To investigate the frequency and outcome of new-borned babies with devices related hospital acquired infections in a tertiary neonatal intensive care unit in South of Hungary.
Methods: The number of central line-days, umbilical line and ventilator days were collected prospectively in the Neonatal Intensive Care Unit of Pediatric Department from University of Szeged between 01 February 2000 and 30 June 2000. Relevant microbiological sampling were used to determine the infecting agents. CDC criteria were applied to define infections. Survival rate, blood stream infections (BSI) and ventilator associated pneumonia (VAP) were analyzed. Device-associated infection rate: number of the device associated infection × 1000/number of device used days.
Results: During the investigated period 95 newborn babies were treated. Results are presented in the following table:

Birth weight category	Number of case	Survival (%)	Daycares	BSI rate	VAP rate
<1000 g	17	8/17 (47.0)	493	22.2	9.7
1001–1500 g	25	23/25 (92.0)	525	8.3	7.7
1501–2500 g	22	21/22 (95.4)	384	0	0
>2500 g	31	27/31 (87.0)	327	0	0
All	95	79/95 (83.1)	1729	13.1	7.7

Conclusion: Survival in the very low birth weight categories (<1500 g) was 73.8% and the device-associated infection rate was high. In the higher birth weight categories the survival was 90,5% and no device-associated infections were detected.

P1407 A multiple-resistant and rare cause of neonatal pneumonia due to *Aerococcus viridans*

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Aerococcus viridans is an aerobic, alpha-hemolytic, Gram-positive cocci with a strong tendency to form tetrads. This organism was frequently found on objects in the delivery room, recovery room, nursery, intensive care unit, patients wards, and delivery suite. It has been isolated from patients with urinary tract infections, endocarditis, bacteremia, and pneumonia. We report a case of *Aerococcus viridans* pneumonia in a newborn who was receiving prophylactic ampicillin and netilmicin treatment for minimally respiratuar distress syndrome. Since respiratory functions were also aggraveted, the quantitative culture of endotracheal aspirate fluid revealed 105 cfu/mL alpha hemolytic streptococcus. This microorganism was resistant to all penicillin MIC values and highly resistant to clarithromycin (MIC values ≥ 2 μg/mL) and cefotaxime (MIC values ≥ 32 μg/mL) and intermediate resistant to meropenem (MIC values ≤ 2 μg/mL). It was suspectible to clindamycin (MIC values $\leq 0{,}16$ μg/mL) and vancomycin (MIC values ≤ 1 μg/mL). To our knowledge, this is the third reported case of infection due to a penicillin resistant strain, and the reported case of infection due to a first multiresistant strain. This case was treated with teicoplanin successfully.

P1408 Plasmids profiles and high-level resistance to mupirocin in *Staphylococcus cohnii* ssp. *cohnii* isolated in the ICU of a pediatric hospital

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Objectives: *S. cohnii* ssp. *cohnii* is known as forming small and transient populations on human skin. However, this species has also been isolated from different kinds of opportunistic infections. Numerous multiresistant isolates were found in the ICU of a pediatric hospital. Among 235 investigated strains 46% presented high level of resistance to mupirocin when tested by the Mup200 discs method. The objective to this study was to characterise plasmids profiles of mupirocin resistant isolates of *S. cohnii* ssp. *cohnii*.
Materials and methods: Isolation of plasmids was performed according to the modified method of Parisi and Hecht. The number and size of plasmids were established by gel agarose analysis of extrachromosomal DNA. The broth dilution method was used to determine MICs, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).
Results: MIC values for mupirocin were established for 33 isolates from our collection. Sixteen of the isolates were susceptible (MIC < 4 μg/mL), 15 possessed high level mupirocin resistance (MIC > 1024 μg/mL) and two showed low level of resistance (MIC $= 8$ μg/mL and MIC $= 128$ μg/mL). Plasmid profiles for these isolates were determined. All of the investigated strains carried plasmids. An average number of plasmids was five, but no less than two plasmids were found. In the high level resistant group there were four isolates carrying small and medium plasmids of size less than 20 kb. Each of the other investigated isolates carried at least one plasmid bigger than 20 kb. The investigated isolates of *S. cohnii* had plasmids varying in size – from 1 to 50 kb. Most of them possessed plasmids of about 7 and/or 4.5 kb but this character did not correlate with the resistance to mupirocin.
Conclusion: Our results show diversity of plasmid profiles of the investigated isolates of *S. cohnii* resistant to mupirocin. In their cells big plasmids about 40 kb can often be found. In *S. aureus*, high resistance to mupirocin genes are located in plasmids of this size. In *S. cohnii* such big plasmids are present in both resistant and susceptible to mupirocin strains.

Nosocomial, abdominal, and gastro-intestinal infections

P1409 More clinical failure and costs due to inappropriate empiric treatment of secondary intra-abdominal infections

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Objective: To better understand the incidence and empiric antibiotic treatment of secondary intra-abdominal infections (IAI) and to assess whether initial empirical antibiotic therapy affects patient outcomes we conducted a population-based retrospective cohort study in the Netherlands.
Methods: Within the PHARMO record-linkage database, we identified all patients hospitalized for secondary IAI between 1995 and 1998. In-hospital antibiotic drug use was obtained from the computerized inpatient pharmacy files. Inspected outcomes were appropriateness of initial empirical antibiotic therapy, clinical failure (second line treatment, re-operation, death), length of stay and costs of hospitalization. Associations between outcomes and treatment were estimated using multivariate logistic and linear regression models.
Results: In the source population of 228 000 persons, 175 cases were classified as secondary IAI (mean age 49.3 ± 24.5, 50.9% male) resulting in an incidence

of 2.3/10 000 person-years (95% CI: 2.0–2.7). Initial antibiotic treatment was appropriate for 84% of the cases. The risk of clinical failure was 17.1%. Inappropriate initial antibiotic treatment increased the risk of clinical failure 3.4-fold (95% CI: 1.3–9.1). The median length of stay was 10 days (IQR: 1–19) and the median cost of hospitalization was € 4131. The length of stay and cost were significantly greater for persons with clinical failure.
Conclusion: This study demonstrates that clinical failure of initial inappropriate antibiotic therapy in secondary IAI patients is substantial and leads to increased length of stay and costs of hospitalization. Inappropriate initial antibiotic therapy is an important independent determinant of clinical failure and should be avoided. Adequate antibiotic treatment should be directed against the polymicrobial nature of secondary IAI.
Acknowledgement: This study was supported by an unconditional grant from Merck & Co, NJ, USA.

P1410 Association between inappropriate initial empiric antibiotic therapy and clinical outcome among patients undergoing surgery for community-acquired intra-abdominal infections in Spain

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Objective: To assess the association between inappropriate initial empiric antibiotic therapy and the clinical outcomes among patients undergoing surgery for community-acquired intra-abdominal infections (IAI) in Spain.
Methods: Records of patients who underwent surgery for community-acquired IAI between the period of 10/1998 and 08/2002 in hospitals in Spain, were reviewed. Initial empiric antibiotic therapy was classified as inappropriate if at least one pathogen was resistant to all antibiotics in initial regimen in case of positive culture, while in case of negative/missing culture, a guideline by Bohnen (1992) was used for classifying appropriateness of initial empiric therapy. Therapy was classified as successful if IAI was resolved with initial therapy or with decrease from initial therapy; as unsuccessful otherwise. Logistic regression analyses were performed to assess associations between appropriateness of therapy and clinical outcomes, after adjusting for patients' characteristics and site/type of infection.
Results: A total of 425 patients were included. Of these patients, 387 (91%) received appropriate initial empiric therapy while rest was on inappropriate therapy. Compared with patients on appropriate therapy, patients on inappropriate therapy were less likely to have success with their initial empiric therapy (79% vs. 26%, $P < 0.01$) and more likely to require additional antibiotic therapy (40% vs. 7%; $P < 0.01$) or being re-hospitalized within 30 days of discharge (18% vs. 3%, $P < 0.01$). Multiple logistic regression after controlling for other factors showed that patients on inappropriate therapy were 10 times less likely to have success in their therapy (OR = 0.096, 95% CI: 0.045–0.206). Also patients on inappropriate initial empiric therapy, were more likely to experience death (OR = 1.2, 95% CI: 0.2–6.0), re-operation (OR = 3.0, 95% CI: 1.3–7.1), or re-hospitalization within 30 days of discharge (OR = 7.1, 95% CI: 2.6–19.2). Confining the analysis only to patients with positive culture ($n = 199$) showed similar results, patients on inappropriate initial empiric therapy were less likely to have success in their therapy (OR = 0.15, 95% CI: 0.06–0.37).
Conclusion: Among patients undergoing surgery for community-acquired IAI, inappropriate initial antibiotic therapy was associated with significantly higher risk of unsuccessful clinical outcomes including death, re-operation, re-hospitalization or requirement for additional parental antibiotic.

P1411 Microbiologic features of nonpostoperative nosocomial intra-abdominal infections

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Objectives: Microbiologic characteristics of nosocomial nonpostoperative intra-abdominal infections (NIA) have never been clearly assessed. In clinical trials, this group of patients is often assimilated to postoperative infections. The aim of this study was to clarify this issue in a representative cohort.
Methods: A multicenter prospective evaluation was performed over a period of 6 months (06/2000–01/2001). All pts who underwent surgery for NIA were included. Clinical and microbiological characteristics were recorded.
Results: Among the 247 included pts (complicated appendicitis (6%) or biliary tract infection (28%) or peritonitis (64%)), samples were taken in 203 pts (82%). At least one organism was cultured in 193 pts. A total of 262 organisms were isolated including 146 (56%) Gram-negative bacilli (GNB), 70 (27%) Gram-positive cocci (GPC), 27 (10%) anaerobes and 16 yeasts (6%). GNB were mostly Enterobacterieaceae (*E. coli* ($n = 86$, 42%)) and the group *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp. ($n = 29$, 14%)) and non fermenting GNB (*P. aeruginosa*, $n = 11$, 5%). GPC were mostly staphylococci ($n = 10$, 5%), streptococci ($n = 25$, 12%) enterococci ($n = 31$, 15%). Polymicrobial infections (70% of the cases) were mostly due to combinations of GNB and GPC ($n = 135$), or GNB and anaerobes ($n = 15$). Bacteremia was reported in 30 pts (12%) recovering 34 organisms (including *E. coli* ($n = 13$), staphylococci ($n = 6$) and bacteroides ($n = 6$)). In 40 pts (20%), at least one organism was resistant to the administered regimen (>2 bacteria in 3 pts), including *E. coli* ($n = 13$), *Enterobacter* spp. ($n = 4$), *Pseudomonas* spp. ($n = 3$), enterococci (five *Enterococcus faecium*) and yeasts ($n = 3$).
Conclusion: Our results give a probably good reflect of microbiological features of NIA. Their assimilation in bacteriological terms to postoperative infections is probably inadequate. Interestingly, proportion of resistant strains toward the administered treatments seems to be low when compared with published reports. In view of our results, antibiotic recommendations elucidated for community-acquired infections could probably be applied to patients with NIA infections.

P1412 Antibiotic therapy in severe acute pancreatitis

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Objectives: Contamination of necrotic foci and peripancreatic fluid collections obvious deteriorate the clinical course of necrotizing pancreatitis and in most patients determine the insufficient outcome of acute pancreatitis. The purulent-septic complications are the main cause of the lethal outcome of the late phase of acute pancreatitis. The rationale antibiotic therapy is the necessity part of the complex management in patients with severe pancreatitis.
Methods and results: Purulent-septic complications were noted in 61 (32.4%) and were the cause of death in 27 (14.4%) patients with necrotizing pancreatitis (188 patients). The following germs determined the purification: *Escherichia coli*, *Proteus vulgaris*, *Enterobacter* spp., *Pseudomonas aerogenosa*, *Staphylococcus spp., Streptobacillus moniliformis, Moraxella lacunata, Pneumococcus, Candida*, and *Bacteroides fragilis*. The monoculture was noted in 49 patients, in 12 – the association of microorganisms. Development of purulent-septic complications was noted in 33.3%, when the monotherapy with cephalosporins of the first generation were applied and only in 16.7% – when the cephalosporins of the third generation were used. Quinolones were applied with combination of metronidazole and in only 16%, this therapy was ineffective.
Conclusion: Thus, the cephalosporins of the third generation and quinolones with metronidazole are effective in the prevention of the purulent complications in patients with necrotizing pancreatitis. Applying of these groups of antibiotics permit to decrease the number of necrosis purification from 37.9–25.9%.

P1413 Etiology of infectious peritonitis in patients undergoing continuos ambulatory peritoneal dialysis (1987–2001)

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Objectives: According to the literature, patients undergoing continuous ambulatory peritoneal dialysis (CAPD) have 0.4–0.6 episodes of peritonitis every 12 month. During a 15-year period, patient and laboratory charts of patients undergoing CAPD were reviewed and looked for episodes of CAPD-associated peritonitis. The objective of the present study was to determine the relative prevalence of different infectious agents recovered from peritoneal fluids during such episodes.
Methods: During the study period (1987–2001), sediments of fluids from dialysis bags sent in for microbiological investigation were cultured on routine media directly and after resuspension with aqua dest. for cell lysis. In addition, filtrates of 100 mL of fluids (until 1995) or 10 mL of fluids inoculated in blood culture bottles (since 1996) were cultured.
Results: A total of 1410 dialysis fluids were investigated because of suspected peritonitis. Bacteria and/or yeast could be recovered from 572 (40.6%) peritoneal fluid specimens collected from 216 patients with 341 culture-positive infectious episodes. The most prevalent species were *S. aureus* (30%) and

coagulase-negative staphylococci (CNS) (28%). In total, Gram-positive bacteria were found in 76%, Gram-negative bacteria in 24%, anaerobic bacteria in 0.6% and yeast in 7% of culture-positive episodes. 6.5% of culture-positive episodes were polymicrobial. Approximately 80% of protracted infections (duration: more than 2 months) occurring in 7% of patients were caused by *S. aureus*.
Conclusions: In contrast to other studies, *S. aureus* was found to be most prevalent species in culture-positive CAPD-related peritonitis, outnumbering CNS.

P1414 *Stenotrophomonas maltophilia* peritonitis in CAPD patients: susceptibility to antibiotics and outcome of the treatment

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S. maltophilia is an environmental Gram-negative bacillus, which has been associated with expanding spectrum of clinical syndrome, particularly in hospitalized and immunocompromised patients. Only a few cases of *S. maltophilia* peritonitis have been reported in patients undergoing continuous ambulatory peritoneal dialysis. We report on four patients with *S. maltophilia* peritonitis during the last 4 years and comment on the susceptibility of the isolated strains to antimicrobial agents, the antibiotic combination administered and therapeutic outcome. All the isolated strains were susceptible to trimethoprim–sulfamethoxazole and to ticarcillin–clavulanate. The three of the four patients responded well to antibiotic treatment and only one of them did not respond to the double antibiotic combination, which necessitated the catheter removal. Risk factors, except the chronic renal failure and the presence of indwelling catheter were not identified. None of the patients developed secondary bacterial or fungal peritonitis due to prolonged antibiotic use. Conclusively, we believe that the early administration of the appropriate antibiotic combination could lead to successful treatment of *S. maltophilia* peritonitis, without removal of the catheter. The replacement of the catheter should be confined only to treatment-resistant cases.

P1415 Activity of selected antimicrobials against obligate anaerobes

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Objectives: The aim of this study was to determine susceptibility of strictly anaerobic strains isolated in clinical laboratory in 2001 to selected antimicrobials.
Methods: Two hundred and twenty-five clinical strains of obligate anaerobes were identified with biochemical tests API 20 A (bioMérieux, France). Susceptibility to antimicrobial agents was determined using ATB ANA strips. CCCA medium was applied for isolation of *C. difficile* strains from fecal samples. Toxins A/B were detected with immunoenzymatic test (TechLab, USA).
Results: A hundred and seventy-one strains of Gram-positive anaerobic bacteria (with dominance of *Peptostreptococcus* genus) and 55 of Gram-negative anaerobes (with prevalence of B. fragilis species) were cultured from clinical specimens. *C. difficile* toxins A/B were detected in 39 stool samples (out of 99) and 28 *C. difficile* strains were isolated. Drugs with highest in vitro activity against Gram-positive anaerobic strains were β-lactam antibiotics combined with β-lactamase inhibitors and imipenem. Gram-negative clinical strains were susceptible to imipenem, metronidazole, ticarcillin/clavulanic acid and piperacillin/tazobactam.
Conclusions: Imipenem and β-lactams combined with β-lactamase inhibitors are still highly active against strictly anaerobic bacteria and should be used in empirical treatment of anaerobic infections.

P1416 Comparison of variant *Clostridium difficile* strains found in different hospitals

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Clostridium difficile is the most important infectious cause of nosocomial diarrhea. Virulent strains can produce different combinations of three toxins, TcdA (toxin A), TcdB (toxin B) and binary toxin (CDT). TcdA and TcdB are recognized as the main virulence factors. Binary toxin producing strains were first described about 10 years ago, but their prevalence and the role of toxin CDT in the pathogenesis are not well known. Variant *C. difficile* strains have altered toxin genes (*tcdA* and *tcdB*), coding for toxins TcdA and TcdB, when compared with the reference strain VPI 10463. They can be divided into 20 groups called toxinotypes (I–XX). Variant strains can produce both TcdA and TcdB (A + B + strains; I–VII, IX, XII–XV, XVIII–XX), only TcdB (A-B+ strains; VIII, X, XVI, XVII) or neither TcdA nor TcdB, yet still have parts of toxin genes present (A-B– strains; XI). Most of the data on variant strains come from studies of two large European *C. difficile* collections from Brussels and Cardiff. Here, we compare variant *C. difficile* strains found in hospitals from different countries. Altogether eight hospitals from three countries were included; Japan (six hospitals), USA (1) and Poland (1). Variant strains were detected with RFLP–PCR-based screening of *tcdA* and *tcdB* and another PCR reaction was used for detection of binary toxin gene *cdtB*. Variant strains were found in all eight hospitals and belonged to nine previously described toxinotypes and to two new toxinotypes. Our results show that A–B+ strains from toxinotype VIII are the most prevalent group of variant *C. difficile* strains, followed by toxinotypes III and IV. However, in a given hospital we found from one to nine different toxinotypes. Variant *C. difficile* strains in these collections are a good indicator for the prevalence of such strains in general, but types and number of variant strains can vary substantially among the hospitals and within a single hospital over different time periods.

P1417 Prevalence of *Clostridium difficile*: diarrhea in university hospital in Warsaw

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Clostridium difficile is the main agent responsible for nosocomial diarrhea worldwide. *C. difficile* infection, as a complication of antibiotic treatment appears especially in adults: elderly patients, hospitalized for longer periods and immunocompromised patients. However, *C. difficile* may be an etiological agents diarrhea in a pediatric units. The aim of our study was to examine the frequency of *C. difficile* and its toxins in fecal samples from patients suffering from antibiotic associated diarrhea (AAD). From 1 January 2002 through 31 December 2002 310 patients adults as well as children were diagnosed in direction of *C. difficile*. Patients were hospitalized: 128 in transplantology unit, 43 in surgery unit, 31 in internal unit, 15 in orthopedic unit, 8 in intensive care unit, 13 in another units (urology, dermatology) and 72 in pediatric units (hematology and gastroenterology). The immunoenzymatic *C. difficile* TOX A/*B*-test (TechLab Inc., Blacksburg, USA) was used to detection a both toxins: toxin A and toxin B. All fecal samples were plates on selective medium (CCCA) and incubated under anaerobic conditions. From 310 fecal samples under investigation 183 (59%) of these samples were positive for *C. difficile* toxins by EIA test (TOX A/B). From all samples 77 *C. difficile* strains were isolated. Surgical (40%), transplanological (50%) and pediatric (60%) cases exhibited *C. difficile* diarrhea most frequently.

P1418 Toxigenicity and clindamycin resistance among *Clostridium difficile* strains isolated from patients with antibiotic associated diarrhea in Poland

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Objective: The toxigenicity and clindamycin resistance of 90 *C. difficile* strains isolated from fecal samples of patients adults and children with associated diarrhea (AAD) was tested.
Methods: Toxin production was investigated in 90 *C. difficile* strains isolated in our laboratory. Toxin A was tested by means a rapid slide immunoassay *C. difficile* A test, Clearview (Basingstoke, Oxoid, UK) and toxins A and B were detected by means of immunoezymatic test *C. difficile* TOX A/*B*-test (TechLab, Blacksburg, USA) and confirmed by PCR with specific primer pairs. Cytotoxicity assay was used to detect toxin B. Antimicrobial susceptibility was tested by means *E*-test to clindamycin, erythromycin, imipenem, metronidazole and vancomycin. The *ermB* gene was detected using PCR.
Results: Sixty-six (63%) *C. difficile* strains from all strains under investigation were shown to be toxin A positive by slide immunoassay and 24 (27%) of all strains were negative. The same 66 strains as well as 24 A-negative strains were positive in *C. difficile* Tox A/*B*-test. All strains were positive in cytotoxicity

assay. From all strains 52 (58%) were highly resistant to imipenem and 36 (40%) were sensitive. All strains were sensitive to metronidazole and vancomycin. In our observations all 24 A-negative B-positive clinical *C. difficile* strains were highly resistant to clindamycin and erythromycin and harbor *ermB* gene.

P1419 Detection of *Clostridium difficile* and its toxin a (TcdA) in stool specimens from hospitalized patients

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Objectives: To evaluate the frequency of *C. difficile* recovery in culture and to determine the frequency of *C. difficile* toxin A detection in stool specimens of patients with nosocomial diarrhea, hospitalized in a tertiary care hospital in Warsaw (1200 beds).
Methods: The study comprised stool samples collected (1998–2002) from adult patients suspected of antibiotic associated diarrhea (AAD), colitis (AAC) or pseudomembranous colitis (PMC), hospitalized in different wards. The identification of cultured *C. difficile* strains was confirmed with a latex agglutination test for *C. difficile* antigen (Becton Dickinson, USA). The presence of *C. difficile* toxin A was assayed using a commercial immunoassay (Oxoid, England).
Results: In total 4435 samples collected during five years (1998–568; 1999–707; 2000–801; 2001–1067; 2002–1292). Out of them 1308 (29.5%) have been culture-positive for *C. difficile* (1998–14.6%; 1999–29.3%; 2000–33.8%; 2001–31.2%; 2002–32.0%). The testing for *C. difficile* toxin A revealed 847 (19.1%) positive samples (1998–28.5%, 1999–17.4%; 2000–23.2%; 2001–17.1%; 2002–15.0%).
Conclusion: We observed an increase in the number of stool samples tested for *C. difficile* and in the number of *C. difficile* culture-positive samples over 5 years. A decrease in the number of *C. difficile* toxin A-positive fecal samples is noted during last two years. This phenomenon may be due to an improved antibiotic policy.

P1420 Value of toxin detection and stool culture of *Clostridium difficile* during an outbreak of CDAD on a geriatric ward

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Objectives: *Clostridium difficile*-associated diarrhea (CDAD) remains the leading cause of nosocomial-acquired diarrhea. Prolonged hospital stay and diagnostic and therapeutic procedures due to CDAD cause additional costs.
Methods: From June to December of 2002 62 cases of diarrhea were reported from a geriatric ward of a clinic for Internal Medicine. *C. difficile* and *Clostridium perfringens* -Toxin ELISA and culture for *C. difficile*, *C. perfringens* and *Staphylococcus aureus* were done on all stool samples. In-vitro susceptibility of *C. difficile* was tested for vancomycin, metronidazole, linezolid, fusidic acid, and tetracycline.
Results: Twenty stools were positive for *C. difficile* toxin using ELISA. All 62 stools were cultured and from 29 samples grew *C. difficile*. *C. difficile* was isolated out of 15 ELISA negative stools. Six ELISA positive samples were negative using culture. Additionally, in 15 stools *S. aureus* and/or *C. perfringens* could be islolated. Four samples contained only *S. aureus* and *C. perfringens*, respectively. Twenty-three stools were negative in all tests. All *C. difficile* isolates were susceptible to vancomycin and metronidazole.
Conclusion: Stool culture for *C. difficile* was shown to be more sensitive than toxin ELISA in this study. Culturing the organisms is the prerequisite for susceptibility testing and epidemiological investigations.

P1421 Parasites in nosocomial diarrhea: are they underestimated?

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Objectives: To validate the role possible pathogens in nosocomial diarrhea including parasites and to develop guidelines for our hospital use of stool cultures.
Methods: We evaluated stool samples of hospitalized patients sent to our laboratory during a 16-month period (2001–2002). The samples were first examined macroscopically and then the patients were questioned and confirmed about the '3-day rule'. A total of 226 samples qualifying all these specifications were first examined by direct wet mount and by addition of a drop of iodine and then inoculated to MacConkey and *Salmonella–Shigella* agar to improve recovery of *Salmonella*, *Shigella* and *Aeromonas* species. Part of the sample was concentrated by sedimentation techniques for the recovery of protozoa, eggs, and larvae. A total of 126 concentrated samples were evaluated for *Cryptosporidium* by Kinyoun's modified acid fast stain and direct fluorescence assay with monoclonal antibody. ELISA was used for detection of *Clostridium difficile* toxin A +B. Samples with heavy yeast colonizations were evaluated to be suspicious for fungal infections.
Results: We did not recover *Salmonella*, *Shigella*, or *Aeromonas* from any of the samples studied. Eleven (4.87%) samples yielded positive for *C. difficile* toxin A + B while *Giardia* cysts and/or trophozoites were diagnosed in 10 (4.42%), *Blastocytis hominis* in 9 (3.98%) and *Cryptosporidium* in 1 (0.79%), heavy yeast colonization in 8 (3.54%) samples. The distribution of patients are given in Table 1.

Table 1 Epidemiological data of the patients

Mean age (years)	43.042 ± 22.378
Median age (years)	42 (0–87)
Sex (F/M)	35/59
Hematology–oncology	24 patients
Surgical units	22 patients
Medical units	41 patients
Intensive care units	7 patients
Prior antibiotic use	90 (95.7%)
History of recent surgery	29 (30.8%)
Immunosuppressive treatment	41 (43.6)

Conclusions: Examination of nosocomial diarrhea samples for ova and parasites is not a part of routine stool evaluation. Our study revealed that in 12.38% of the cases, protozoon were the only relevant agents causing nosocomial diarrhea. We found *Cryptosporidium* oocysts in one case with nosocomial diarrhea. In conclusion, the yield of in-hospital stool cultures for enteropathogenic bacteria other than *C. difficile* performed less than 72 h after admission is low. On the other hand, stool specimens of nosocomial diarrhea cases should be examined for parasites in endemic areas.

P1422 Current trends in perioperative antibiotic prophylaxis in colorectal surgery in Russian hospitals

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Objectives: To evaluate the prescribing patterns of surgeons for perioperative antibiotic prophylaxis (PAP) in colorectal surgery (CS) in Russian hospitals.
Methods: The multicenter retrospective study of PAP in CS in Russian hospitals was carried out in 16 centers where case histories of patients who underwent CS in 1999 were analyzed. In all centers 50 consecutive case histories were evaluated. Collected data included demographic information; operation issues; dosage, route and course duration for prescribed antibiotics (pre- and postoperation antibiotic therapy, PAP); surgical site infections (SSI); duration of stay.
Results: Altogether 643 case histories of patients were included in the final analyzes (49% males and 51% females, mean age 50.0 ± 17.4 years). The most common operations were: hemicolectomy – 21%, rectum resection – 19.3%, sigmoid resection – 16.6% and colostomy 13.1%. Overall, PAP was used in 65.5% of all colorectal operations. Cefazolin, gentamicin, cefuroxime, cefotaxime, amoxicillin/clavulanate, metronidazole were the most commonly prescribed antibiotics (18.8, 18.3, 17.7, 8.6, 7, and 5.9% of total prescriptions, respectively). Antibiotics in postoperative period were prescribed in 95% of patients and course was 7 ± 3.3 days. Gentamicin (28.8%), metronidazole (11.9%), cefazolin (11.4%), and ampicillin (11.25) were prescribed mostly. Overall rate of SSI for CS was 13.9%, and it varies from 8.9% after colostomy to 20.2% after rectum resection.
Conclusions: Rate of PAP in CS is low. Antibiotics used for PAP are often inconsistent with recent recommendations. Despite of SSI presence or absence and PAP, antibiotic therapy in postoperative period is common. The guidelines for PAP in CS should be implemented and more education on the subject is necessary.

Quinolones

P1423 BIVEMOX: a Belgian in-vitro evaluation of moxifloxacin

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Objectives: The three most isolated bacteria in community-acquired pneumonia (CAP) and acute exacerbation of chronic bronchitis (AECB) are *S. pneumonia* (SP), *H. influenzae* (HI) and *M. catarrhalis* (MC). A study was performed in Belgium to assess the in-vitro activity of moxifloxacin on clinical isolates of these bacteria in respiratory tract infections including CAP and AECB from ambulatory and hospitalized patients by determination of the MICs using the *E*-test and to compare these results against nine other antimicrobial agents. Additionally, the microdilution method was used to evaluate the susceptibility of SP to moxifloxacin and levofloxacin.
Material and methods: Nine Belgian university and nonuniversity medical microbiology laboratories participated during one winter period (2000–2001 or 2001–2002) in a multicenter in-vitro evaluation of moxifloxacin. Only the Belgian reference center for SP (J. Verhaegen, Leuven) participated during two consecutive winter periods. Each laboratory included strains of SP, HI and MC. The total number of evaluable strains was 1172.
Susceptibility testing: *E*-test: susceptibility testing was performed in the participating centers by using the *E*-test. The antibiotics tested were penicillin, ampicillin, amoxi/clav., doxycycline, clarithromycin, cefuroxime, ceftriaxone, ciprofloxacin, levofloxacin and moxifloxacin. SP (all antibiotics): Mueller–Hinton 5% blood; 0.5 Mc Farland 24 h, HI (all antibiotics): HTM agar; 0.5 Mc Farland 24 h, MC (all antibiotics):Mueller–Hinton 7% blood; 0.5 Mc Farland 24 h. Incubation was done for 24 h in 5% CO_2. Microdilution method: according the NCCLS guidelines, with 5% lysed horse blood. Quality control: SP ATCC 49619; HI ATCC 49766.
Results: see Table 1.

Table 1 MICs (mg/L)

	S. pneumonia		*H. influenzae*		*M. catarrhalis*	
Antibiotic	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Penicillin	0.03	0.5	0.25	64	32	64
Ampicillin	0.008	1	0.25	32	2	16
Amox/clav.	0.008	0.5	0.5	1	0.12	0.25
Cefuroxime	0.03	1	1	2	1	2
Ceftriaxone	0.015	0.25	0.008	0.015	0.25	1
Doxycycline	0.12	8	1	1	0.25	0.5
Clarithromycin	0.12	256	16	32	0.12	0.5
Ciprofloxacin	0.5	1	0.015	0.03	0.03	0.06
Levofloxacin	0.5	1	0.03	0.03	0.06	0.06
Moxifloxacin	0.12	0.25	0.03	0.06	0.06	0.06

Conclusion: This Belgian multicenter study demonstrated the excellent in-vitro potency of moxifloxacin against clinical isolates of SP, HI and MC. Moxifloxacin showed in-vitro with the *E*-test to be 4-fold more active against SP than levofloxacin and demonstrated an equal in-vitro activity against other bacteria. No resistant strains against moxifloxacin were found. The microdilution method confirms the better in-vitro activity of moxifloxacin vs. levofloxacin for SP. Follow-up surveys will be needed to monitor the ecological impact of increased fluoroquinolone usage in respiratory tract infections in Belgium on the susceptibility to moxifloxacin in the coming years.

P1424 Pharmacodynamics of moxifloxacin and levofloxacin against *Streptococcus pneumonia*

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Objectives: To compare the bactericidal activity of moxifloxacin (MXF) and levofloxacin (LEV) against *S. pneumonia* (Spn) at the focal concentrations of MXF and LEV in the respiratory tree in parallel to serum.

Methods: MFX and LEV concentrations in serum (S, C_{max} 3.3/6.6 mg/L), bronchial mucosa (BM, C_{max} 5.5/8.3 mg/L), epithelial lining fluid (ELF, C_{max} 24.4/10.9 mg/L), and alveolar macrophages (AM, C_{max} 62.0/52.9 mg/L) following a single oral dose of 400 and 500 mg, respectively, were simulated in a one compartment in vitro pharmacokinetic model. The colony-forming units (cfu) were determined immediately after inoculation (106 cfu/mL) and after incubation for 1–24 h at 37°C. Three Spn strains were used: pen. sens. (4241), pen. res./cip. sens. (PP62), and a lab. generated pen. res., 1st step quin. res. strain (19397).
Results: Times to achieve a 99.9% kill are summarized in the table:

Strain no.	Drug	S	BM	ELF	AM
4241	MXF	6.00	6.00	6.25	6.00
	LEV	6.75	6.00	6.00	6.00
PP62	MXF	2.91	2.64	1.11	3.06
	LEV	4.03	2.64	5.14	4.58
19397	MXF	>24	5.28	5.55	5.14
	LEV	>24	>24	>24	9.17

Conclusions: MXF was rapidly bactericidal against all test strains including the first step quinolone resistant strain and exhibited enhanced pharmacodynamics compared with levofloxacin.

P1425 Comparative bactericidal activity of moxifloxacin, gatifloxacin, and levofloxacin using simulated ELF concentrations

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Objectives: The fluoroquinolones are bactericidal agents frequently used in the treatment of respiratory tract infections. In this study, we compared the killing kinetics of moxifloxacin (MXF), gatifloxacin (GAT), and levofloxacin (LEV), against fluoroquinolone susceptible and resistant *Streptococcus pneumonia* (Sp) at concentrations typically reached in the epithelial lining fluid (ELF).
Methods: Six strains of Sp., two fluoroquinolone susceptible, two *parC* mutants, and two *parC/gyrA* mutants were tested using Mueller–Hinton broth supplemented with 5% lysed horse blood. Cultures were inoculated at a density of 1×10^6 cfu/mL, incubated aerobically at 35°C, and tested for viable growth at 0, 1, 2, 4, 6, 12, and 24 h. Simulated ELF concentrations of MXF (400 mg), GAT (400 mg), LEV (500 mg), and LEV (750 mg), adjusted for protein binding, were 12, 5.6, 7.4, and 14 µg/mL, respectively. Protein binding of MFX, GAT, and LEV were taken to be 50, 20, and 30%, respectively.
Results: All agents were rapidly bactericidal against the susceptible strains of Sp. A 3-log drop in cfu was observed within 2–4 h for all agents. MXF achieved complete eradication within 6 h, while GAT and LEV achieved complete eradication within 12 h. For the *parC* mutants (strains #9177 and #9427), MXF achieved a 3-log kill at 3 h, GAT and LEV (750) at 4 h, and LEV (500) at 7 h. Complete eradication with MXF was seen at 6 h with strain #9177 and 12 h in strain #9427. Both GAT and LEV (750) eradicated strain # 9177 at 12 h, however, re-growth was observed in the culture treated with LEV (500) between 12 and 24 h. Repeat susceptibility testing of this isolate demonstrated a 4-fold increase in the LEV MIC. Both GAT and LEV eradicated strain #9427 at 24 h. Complete eradication was not observed with any agent in the *parC/gyrA* mutants. Only MXF demonstrated a 3-log cfu decrease. GAT and LEV (750) were bacteriostatic over 24 h, while LEV (500) had growth kinetics similar to the growth control.
Conclusions: All the fluoroquinolones are extremely active against susceptible Sp. Superior killing kinetics were observed with the 8-methoxy fluoroquinolones (MXF and GAT), compared with LEV against low level fluoroquinolone resistant Sp. Only MXF maintained its bactericidal activity against high level fluoroquinolone resistant strains of Sp.

P1426 In vitro activity of moxifloxacin compared with other quinolones against clinical isolates of *Streptococcus pneumonia*

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Objectives: To determine the in vitro activity of moxifloxacin in comparison with some quinolones such as levofloxacin, sparfloxacin, and ofloxacin, against *Streptococcus pneumonia* isolates.
Methods: A total of 66 clinical isolates of *S. pneumonia* were investigated. A part of them ($n = 30$) were isolated during 1999–2000 and the others ($n = 36$) in 2001–2003. The antibiotic susceptibility tests were performed by standard disc-diffusion method and the results were interpreted according to NCCLS recommendations. Penicillin resistance was detected by *E*-test method (AB Biodisk, Sweden).
Results: The rate of penicillin nonsusceptible strains was 45.4%, and three of them (4.5%) had high level resistance which were isolated in 2001–2003. The rate of moxifloxacin susceptibility were 98.4%. Levofloxacin and sparfloxacin susceptibility among *S. pneumonia* isolates were found as 93.9%, and 90.9% of isolates were susceptible to ofloxacin.
Conclusion: Newer fluroquinolones had good activity against *S. pneumonia* strains, particularly in the era of emerging penicillin resistance. These results show that moxifloxacin could be considered as an effective alternative among this group of antibiotics against pneumococcal infections.

P1427 Gatifloxacin activity on slow growth phase bacteria

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Background: Persistence of microorganisms in chronic infections is due to modification of metabolism and biofilm formation. It is well demonstrated in case of cystic fibrosis and bone infection, and was recently observed in *Streptococcus pneumonia* (SP) chronic otitis media.
Objectives: To study bactericidal activity of gatifloxacin (GTF), a new fluoroquinolone and betalactams on bacteria in different growth phase.
Methods: Bactericidal activity of GTF and ceftazidime was determined in broth and phosphate buffer for *Pseudomonas aeruginosa* (1), *Escherichia coli* (2). Killing curves were also performed with GTF and oxacillin on *Staphylococcus aureus* (5) and *S. epidermidis* (1). Each experiment was performed twice at concentrations 2-, 4-, 8-fold the MIC and bactericidal activity was defined as a decrease of 3 log CFU/mL. For 5 SP, opaque (O) and transparent (T) variants of each strain were first isolated. Killing curves were performed in brain broth with amoxicillin and GTF.
Results: In phosphate buffer, GTF kept its bactericidal activity on BGN while a decrease of only 1.5–2 log was observed on SA and SE. Ceftazidime, oxacillin lost their bactericidal activity on all tested bacteria in buffer. An increase of 8–32-fold MIC was necessary with amoxicillin to be bactericidal on opaque SP variants while GTF had the same bactericidal activity on T and O variants.
Conclusion: For SP increase in concentrations of amoxicillin is necessary to reach bactericidal activity on opaque variants while there is no activity of batalactam antibiotics on BGN and staphylococci in slow growth phase. With GTF a bactericidal activity was observed on BGN and SP even in slow growth phase. These results may be important to consider to avoid therapeutic failures in chronic situations.

P1428 Cross-predictability of gatifloxacin and levofloxacin susceptibility in vitro of clinical enteric isolates

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Objective: Antibiotic formulary changes often require a similar laboratory change in antibiotics tested to ensure accurate susceptibility (SPT) reporting. There can be a long time delay for new agents to appear on commercial SPT panels which can necessitate manual testing of the new agent as occurred at our institution with gatifloxacin (GAT). This study was designed and performed to determine if levofloxacin (LEV) SPT from MicroScan correlated with GAT SPT determined by *E*-test methodology against aerobic Gram-negative enterics.
Methods: Over 1100 clinical isolates of Enterobacteriaceae were tested for their SPT to LEV and GAT. Included in the SPT testing were: *Citrobacter koseri* (#66); *E. coli* (#730); *Enterobacter aerogenes* (#37); *E. cloacae* (#63); *Klebsiella pneumonia* (#105); *Proteus mirabilis* (#144); *Serratia marcescens* (#14); and *Shigella sonnei* (#5). For LEV SPT to each isolate was tested using Gram-negative combo panels in the MicroScan WalkAway system. For GAT SPT of each isolate was tested on the same day using *E*-test methodology. Interpretation of SPT results was performed to determine discrepancy rates using NCCLS guidelines for susceptible (S), intermediate (I), and resistant (R) categories.
Results: Both GAT and LEV showed excellent activity against the isolates tested. The categorical discrepancy rate between the two agents was 1% (12 of 1164 comparisons). Discrepancies were distributed among the isolates as follows: *C. koseri,* 1; *E. coli,* 4; *E. cloacae,* 4; *K. pneumonia,* 2; and *P. mirabilis*, 1. Results indicated four major discrepancies (S to GAT and R to LEV) and occurred in one isolate each of *C. koseri*, *E. coli*, *E. cloacae*, and *K. pneumonia*. The remaining discrepancies were considered minor and were as follows: 1 S to GAT and I to LEV; 3 I to GAT and R to LEV; 3 I to GAT and S to LEV; and 1 R to GAT and I to LEV. Based on overall results major and minor discrepancies occurred in 0.3 and 0.7% of isolates, respectively.
Conclusions: This study found that categorical SPT results for Enterobacteriaceae with GAT and LEV were comparable when tested by two different methodologies. The overall discrepancy rate was acceptable (1%) and these results support the use of LEV to predict SPT to GAT among Enterobacteriaceae.

P1429 Contemporary activity of grepafloxacin: re-evaluation of antimicrobial features of a potent fluoroquinolone

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Objective: To re-evaluate the potency and usable spectrum of activity for grepafloxacin against contemporary pathogens collected from clinical infections in 2001–2002. These results will update the grepafloxacin role compared with other quinolone agents introduced since 1999, in preparation for expanded in vitro and in vivo investigations against resistant (R) strains.
Methods: A total of 995 strains of recently isolated bacterial pathogens were tested by reference NCCLS methods compared with 25 other agents including four marketed fluoroquinolones (FQ). The organisms included: *Escherichia coli* (EC; 52), *Klebsiella pneumonia* (KPN; 51), *Enterobacter cloacae* (ECL; 55), *Pseudomonas aeruginosa* (PSA; ciprofloxacin-R, 52; and ciprofloxacin-S, 50), methicillin-R *S. aureus* (MRSA; 104), methicillin-susceptible (S) *S. aureus* (MSSA; 58), coagulase-negative staphylococci (CoNS; 50), beta-hemolytic streptoccci (BHS; 52), *Streptococcus pneumonia* (SPN; 167), *Haemophilus influenzae* (HI; 105), *Moraxella catarrhalis* (MCAT; 91) and *Legionella pneumophila* (35).
Results: Grepafloxacin activity was comparable to ciprofloxacin, levofloxacin and gatifloxacin against EC, KPN and ECL (MIC_{90}, 0.03–2 μg/mL; R = 0.0–7.7%). For PSA, grepafloxacin was active against ciprofloxacin-S isolates (MIC_{90}, 2 μg/mL), but not ciprofloxacin-R (MIC_{90}, >8 μg/mL). Against MSSA, grepafloxacin S rate was 91.4%, equal to levofloxacin; none of the FQs were active against MRSA or CoNS. Gatifloxacin and grepafloxacin had the same MIC_{90} vs. BHS (0.25 μg/mL) and penicillin-susceptible SPN (0.25 μg/mL). Grepafloxacin and other FQ activities were not influenced by penicillin R in SPN. Grepafloxacin was very active against HI (MIC_{90}, 0.03 μg/mL), MCAT (0.03 μg/mL) and *Legionella* spp. (0.5 μg/mL).
Conclusions: These recent results indicate that grepafloxacin has retained its potent spectrum against Enterobacteriaceae, methicillin-S staphylococci, and the pathogens causing community-acquired respiratory tract infections. Additional potency was observed vs. ciprofloxacin-S PSA and other streptococci. As issues of adverse drug reactions are more accurately evaluated and minimized, it remains clear that grepafloxacin continues to be an excellent candidate FQ for ambulatory care practice settings.

P1430 Antianaerobic activities of garenoxacin, gatifloxacin, moxifloxacin compared with those of levofloxacin, ciprofloxacin, clindamycin, metronidazole and two beta-lactams

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Objectives: MICs were determined on garenoxacin (GAR) moxifloxacin (MOX), gatifloxacin (GAT), levofloxacin (LEV), ciprofloxacin (CIP), coamoxyclav (AMC), piperacillin–tazobactam (PTZ), clindamycin (CLN), imipenem (IMI) and metronidazole (MOL) against 175 anaerobes isolated from human clinical samples.
Methods: Reference agar dilution (standard M11 A5, NCCLS).
Results: GAR, MOX, GAT, LEV and CIP, inhibited at 4 mg/L, respectively, 100, 94, 93, 82, and 44% of the *Bacteroides fragilis* group strains whereas resistance rates were, respectively: AMC 1%, PTZ, IMI and MOL 0%, CLN 35%. Considering the three fluoroquinolones, the $MIC_{50/90}$s (mg/L) can be seen in the table.

Microorganisms (*n*)	GAR	MOX	GAT	LEV	CIP
B. fragilis group (71)	0.25/1	0.5/4	0.5/4	2/16	8/64
Other Gram-negative bacilli (44)	0.12/0.5	0.25/2	0.25/2	0.5/4	1/8
Clostridia (15)	0.25/1	0.25/2	0.5/1	1/4	1/16
Non-sporulated Gram-positive rods (22)	0.25/1	0.25/1	0.5/1	0.25/2	0.5/16
Peptostreptococcus spp (23)	0.12/0.12	0.25/2	0.5/1	0.5/4	1/2
All anaerobes (175)	0.25/1	0.25/2	0.5/4	1/8	2/32

GAR, MOX, and GAT unlike other fluoroquinolones demonstrated high activity against the *B. fragilis* group and clostridia but GAR was the more potent fluoroquinolone. GAR, MOX, and GAT were also more potent than LEV and CIP against gram positive rods and most anaerobic cocci. At concentration of 2 mg/L, GAR inhibited all strains of *Fusobacterium, Porphyromonas, Propionibacterium* and Gram-positive cocci. At concentration of 2 mg/L, GAR inhibited all Gram-positive anaerobes (60 strains) and 103 out of 105 Gram-negative anaerobes. Overall 175 anaerobes, GAR inhibited 99 and 100% of the strains investigated at concentrations of 2 and 4 mg/L, respectively. Comparatively, at the same concentrations, moxifloxacin inhibited 94 and 98% and GAT inhibited 91 and 96% of the whole anaerobes. Overall clindamycin resistance rate was 18.5%.
Conclusion: Only GAR, MOX, and GAT were able to inhibit at least 95% of the anaerobes investigated. MOX and GAR had similar activities but GAR was the more potent agent. Their broad anaerobic spectrum demonstrated in vitro is very promising to treat anaerobic or mixed infections; further clinical evaluations are needed.

P1431 In vitro activity of garenoxacin, moxifloxacin, and gatifloxacin against *Bacteroides fragilis* group isolates with varying degrees of resistance to carbapenems

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Objectives: Antimicrobial resistance among the Bfg has increased to beta-lactams, clindamycin, and now metronidazole therefore new antimicrobial options are needed. Resistance to Cbs particularly imipenem (IM), results in cross-resistance to all beta-lactams thus limiting the number of therapeutic options. Newer quinolones exhibit anti-anaerobic activity but little information details activity against Cb-resistant isolates. Here, we compare the activity of GRN, MXF, and GAT against a select group of Cb-resistant and -susceptible Bfg isolates.
Methods: Sixty-five isolates of the Bfg previously tested by broth microdilution assays were used. Of these, 21 had high level (MICs 32 mg/L) IM resistance and were cross-resistant to ertapenem (ERT) (100%), cefoxitin (FOX) (100%), ticarcillin–clavulanate (T-C) (100%), and piperacillin–tazobactam (P-T) (100%), and clindamycin (CL) (62%). The remaining 44 isolates were susceptible to IM (MICs 4 mg/L) with 11 of the 44 with ERT MICs 8 mg/L. All 44 were susceptible to P-T and had varying degrees of susceptibility to FOX, T-C, P-T, and CL. MICs were determined to GRN, MXF, and GAT using recommended *E*-test methodology. MICs were collated to include: MIC range, mode MIC, MIC_{50}, MIC_{90}, and percentage of isolates inhibited at potential susceptible breakpoints of 1, 2, and 4 mg/L.
Results: MIC results indicated varying degrees of activity of GRN, MXF, and GAT for the Bfg overall. The mode MIC, MIC_{50}, MIC_{90}, and percentage inhibited at 1, 2, and 4 mg/L for the overall group were: GRN: 0.125, 0.38, 32, 83%, 83%, 86%; MXF: 0.38, 0.38, 32, 74%, 77%, 82%; GAT: 0.5, 0.79, 32, 60%, 72%, 77%. Comparison of IM-susceptible or-resistant isolates showed equal activity for GRN, MXF, and GAT against the two groups; at 2 mg/L GRN inhibited 81% vs. 84%; MXF 77% vs. 76%, and GAT 62% vs. 77%, respectively. Cross-resistance (MIC's 8 mg/L) to GRN, MXF, and GAT occurred in nine isolates.
Conclusions: Although, still at a low rate, increased reports of Cb resistance in the Bfg pose a therapeutic challenge due to concurrent cross-resistance to other beta-lactams. These data show that GRN, MXF, and GAT exhibit potent but varying rates of activity against a select group of Bfg isolates. The activity of these agents is independent of Cb (beta-lactam) resistance and would appear as potential therapeutic options for monotherapy of mixed aerobic–anaerobic infections.

P1432 Comparative activity of Garenoxacin against ciprofloxacin-susceptible and -resistant *Streptococcus pneumonia* isolates: Report from the SENTRY Antimicrobial Surveillance Program (Europe, 1999–2001)

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Objective: To comparatively study the activity of garenoxacin, a new desfluoro (6) quinolone, in ciprofloxacin susceptible and resistant *S. pneumonia* isolates.
Methods: A total of 1613 *S. pneumonia* isolates causing respiratory tract infections were collected in 30 laboratories from 14 European countries during the SENTRY Antimicrobial Surveillance Program from 1999 to 2001. Susceptibility testing (microdilution, NCCLS) was centralized (The Jones Group, Iowa).
Results: Overall ciprofloxacin resistance (MIC $\geq$4 mg/L) was 3.8%, 0.4% for levofloxacin and gatifloxacin, and 0.2% moxifloxacin, but nonexistent for garenoxacin. During the studied period, ciprofloxacin-resistant isolates were concentrated in France (24.6%), Italy (24.6%), Poland (8.2%), Ireland (8.2%), Spain (6.5%), and UK (6.5%). Quinolone activity in ciprofloxacin-susceptible and -resistant isolates is shown in the table.

		Ciprofloxacin-S[a] (96.2%)	Ciprofloxacin-R[b] (3.8%)
Levofloxacin[c]	Range	$\leq$0.12–2	0.5 to >4
	$MIC_{50/90}$ (R%[b])	1/1 (0.0%)	1/4 (11.5%)
Gatifloxacin[c]	Range	$\leq$0.03–1	0.25–4
	$MIC_{50/90}$ (R%)	0.25/0.5 (0.0%)	0.5/1 (9.8%)
Moxifloxacin[c]	Range	$\leq$0.03–0.5	0.12–2
	$MIC_{50/90}$ (R%)	0.12/0.12 (0.0%)	0.25/0.5 (5.1%)
Garenoxacin[d]	Range	$\leq$0.03–1	$\leq$0.03–0.5
	$MIC_{50/90}$ (R%)	0.06/0.06 (0.0%)	0.06/0.12 (0.0%)

S: susceptible; I: intermediate; R: resistant. [a]$\leq$2 mg/l; [b]$\geq$4 mg/l; [c]I + R, NCCLS criteria, [d]gatifloxacin criteria were used for garenoxacin.

Conclusions: Garenoxacin was the most active quinolone tested against *S. pneumonia* isolates. Although, 11.5% of ciprofloxacin-resistant *S. pneumonia* isolates were also resistant to levofloxacin, 9.8% to gatifloxacin, and 5.1% to moxifloxacin, no garenoxacin resistant isolates were observed in Europe. Waiting for clinical studies, these results validate the potential utility of this compound for the treatment of *S. pneumonia* respiratory tract infections in this region.

P1433 In vitro activity of gatifloxacin and seven other antibiotics against respiratory and urinary tract pathogens from the community: results of the BASIC study

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Objectives: To obtain present figures on resistance towards gatifloxacin and other antibiotics.

Methods: A total of 23 centers in Austria, Belgium, France, Germany, Italy, Portugal, Spain, and Switzerland are involved in the Bacterial Annual Susceptibility Information Collection (BASIC) study. The MICs of gatifloxacin (Gati), ciprofloxacin (Cipro), clarithromycin (Clari), benzylpenicillin (Pen), amoxicillin (Amox), amoxicillin/clavulanic acid (Coamox), cefuroxime (Cur) and cefixime (Cix) were determined using the microdilution method. Each center is requested to investigate about 30 strains each of the following species: *S. pneumonia* (Spn), *S. pyogenes* (Spy), *S. aureus* (Sau), *E. faecalis* (Efa), *M. catarrhalis* (Mca), *H. influenzae* (Hin), *E. coli* (Eco), *K. pneumonia* (Kpn), *P. mirabilis* (Pmi) and *P. aeruginosa* (Pae).
Results: In 2001 and 2002, altogether 5462 strains were enrolled. Apart from deviations in some regions important MIC_{90}%/percentage susceptible were as follows:

Table 1

	n	Gati	Cipro	Clari	Coamox	Cur-axetil
Spn	558	0.5/98.9	2/–*	≥64//67.7	1/98.0	4/79.4
Hin	580	0.03/100	0.03/99.8	16/86.0	1/95.3	2/94.8
Mca	361	0.06/100	0.06/99.7	0.5/99.2	0.25/98.9	2/97.8
Sau	589	2/92.0	16/80.5	≥64/68.1	≥16/86.1	≥64/86.8
Eco	670	2/91.3	1/90.0	≥64/–*	≥16/75.2	8/95.2
Pmi	563	4/88.6	2/89.0	≥64/–*	≥16/87.6	32/85.6
Pae	569	16/72.1	16/76.3	≥64/–*	≥16/–*	≥64/–*

*NCCLS breakpoints not available.

Conclusion: From the oral antibiotics tested gatifloxacin has the highest activity and broadest spectrum against all relevant respiratory and urinary tract pathogens. Gatifloxacin is a promising alternative for therapy of respiratory and urinary tract bacterial infections.

P1434 In vitro activity and postantibiotic effect of ABT-492 and other antimicrobial agents against *Legionella*

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Background: ABT-492 is a new potent quinolone agent which has enhanced activity against respiratory pathogens. The in vitro activity and the postantibiotic effect (PAE) of ABT-492, moxifloxacin, gatifloxacin, levofloxacin, ofloxacin, ciprofloxacin and erythromycin were evaluated against isolates of *L. pneumophila* and isolates of *L.* other than *pneumophila*.
Methods: MIC values were determined by standard agar dilution procedure (NCCLS) using buffered yeast extract agar. The PAE was determined by exposing the isolates to the test antimicrobial for 1 h at 4 times the MIC. The antibiotic was removed by consecutive centrifugation. The PAE was calculated by measuring bacterial growth kinetics in similar antimicrobial-free cultures.
Results: The species tested included *L. pneumophila* serogroups 1–12 (300 isolates), *L. dumoffii* (30), *L. micdadei* (30) and *L. longbeacheae* (25). ABT-492 ($MIC < 0.004$ mg/L) was superior to levofloxacin and gatifloxacin (MIC_{90} 0.016 mg/L) and significantly ($P < 0.001$) more active than moxifloxacin (MIC_{90} 0.03 mg/L), ciprofloxacin (MIC_{90} 0.03 mg/L) and ofloxacin (MIC_{90} 0.06 mg/L), against *L. pneumophila*. *L. pneumophila* serogroups 2–4, 6–9 and 12 strains ($MIC_{90} < 0.004$ mg/L) were more susceptible than *L. pneumophila* serogroups 1 and 5 (MIC_{90} 0.03 mg/L). Against *L. micdadei* and *L. dumoffii*, erythromycin (MIC_{90} 1 mg/L) was less active than ABT-492 ($MIC_{90} <$ 0.004 mg/L) and levofloxacin (MIC_{90} 0.016 mg/L). Against *L. longbeacheae*, ABT-492 (MIC_{90} 0.016 mg/L) was as active than levofloxacin and was more active than moxifloxacin, gatifloxacin, ofloxacin and ciprofloxacin (MIC_{90} 0.03 mg/L). Against ERY-resistant *L. pneumophila*, ABT-492 showed a PAE at >24 h, compared with 3.3 h or less with quinolones tested. Against ERY-susceptible *L. pneumophila*, a PAE of 24 h was also observed for ABT-492. For ERY-resistant *Legionella* spp. other than *L. pneumophila*, ABT-492 had PAEs in excess of >24 h, which was significantly longer than PAE of tested quinolones (<4.1 h).
Conclusion: Owing to excellent in vitro activity and significant PAE of ABT-492, additional studies must be performed to determine the in vivo activity of this agent for the treatment of *Legionella* infections.

P1435 Predictive value of nalidixic acid resistance in detecting *Salmonellas* with decreased ciprofloxacin susceptibility

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Objective: Over the last few years there has been a dramatic increase in the incidence of human Salmonellosis with nontyphoidal serotypes in developed countries. Only a few cases of treatment failure due to *Salmonella* strains (including *S. typhi*) that are resistant to nalidixic acid and strains exhibiting decreased fluoroquinolone susceptibility have been reported. The purpose of this study was to determine resistance to nalidixic acid could be used to screen for decreased ciprofloxacin susceptibility of our Salmonella strains of community origin.
Methods: Seventy-three *Salmonella* strains isolated from stool samples between January 2000 and October 2001 were serotyped according to standard procedures. Antimicrobial susceptibility tests were performed on Mueller–Hinton agar (Difco, USA) using disk diffusion method and on Mueller–Hinton broth (Difco, USA) using broth macrodilution method. *Escherichia coli* (ATCC 25922) was used as a reference standard.
Results: Eight different serotypes were identified among the 73 *Salmonella* isolates studied. All 73 isolates were classified as ciprofloxacin susceptible (MIC, ≤1 mg/mL) according to NCCLS recommendation and 9 out of all isolates were found to be nalidixic acid resistant (MIC, ≥32 mg/mL) by broth macrodilution method. Isolates divided into two major groups. First group with the ciprofloxacin MICs ranging from 0.00025 to 0.008 mg/mL for nalidixic acid susceptible population and the second group with the ciprofloxacin MICs ranging from 0.004 to 0.032 mg/mL for the nalidixic acid resistant population. There was a 3–4-fold increase in ciprofloxacin MICs of nalidixic acid resistant isolates than susceptible ones. This difference was statistically different ($P < 0.001$).
Conclusion: We did not observe typical strains resistant to ciprofloxacin for both nalidixic acid susceptible and resistant strains. MICs for ciprofloxacin were under NCCLS breakpoints for susceptibility. This low level fluoroquinolone resistance may be clinically important. Vasallo et al. reported cases with treatment failure with ciprofloxacin in *Salmonella* infections even the strains are found to be susceptible according NCCLS breakpoints. These strains can never be recognized in laboratories when NCCLs recommendations are accepted as breakpoints. In Turkey, *Salmonella* strains are still susceptible to quinolones. But there are reports showing nalidixic acid resistance ranging between 0.5 and 2.5%.

P1436 Detection of decreased fluoroquinolone susceptibility by use of nalidixic acid disk as screening test in *Salmonella* spp. isolates from children with diarrhea

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Objective: Several treatment failures with ciprofloxacin (CIP) and other fluoroquinolones have been reported in infections due to *Salmonella* spp. The involved isolates had MICs for CIP ≥ 0.125 μg/mL and have been characterized as isolates with decreased susceptibility. The aim of this study was the detection of decreased susceptibility to CIP in *Salmonella* spp., as well as the validation of resistance to nalidixic acid (NAL) disk as screening test.
Material and methods: During one year period (03/2000–02/2001) a total number of 190 *Salmonella* spp. strains from stool specimens of children with gastroenteritis were tested. Among them, 122 (64%) were *S. enteritidis*, 51 (27%) *S. typhimurium* and one *S. typhi*. The remaining isolates were classified to other serogroups. Susceptibility testing was performed by the disk diffusion method with NAL 30 μg and CIP 5 μg disks according to NCCLS criteria. MICs were determined by *E*-test.
Results: All isolates were susceptible to CIP (inhibition zone diameter ≥21 mm, MIC ≤ 1 μg/mL), and 160 isolates (84%) were susceptible to NAL (inhibition zone diameter ≥19 mm, MIC ≤ 16 μg/mL). The resistant strains were typed as follows: *S. enteritidis*, 25; *S. typhimurium*, 2; *S. hadar*, 2; and *S. typhi*, 1. MICs of CIP ranged from 0.008 to 0.5 μg/mL. Twenty-eight isolates had decreased susceptibility to CIP with MIC ≥ 0.125 μg/mL. The

isolates were divided into two populations based on NAL susceptibility: NAL-susceptible with MICs of CIP ranging from 0.008 to 0.032 μg/mL and NAL-resistant with MICs of CIP ranging from 0.064 to 0.5 μg/mL. The mean inhibition zone diameters around CIP of NAL-susceptible and NAL-resistant isolates were 31.5 and 25 mm, respectively. The difference in the mean values was statistically significant. When MIC $\geq$ 0.125 μg/mL was selected as breakpoint for decreased susceptibility to CIP, screening for resistance to NAL disk showed sensitivity 96% and specificity 98%. When MIC of CIP $\geq$ 0.5 μg/mL was selected as breakpoint, screening for resistance to NAL disk showed sensitivity 100% and specificity 98.3%.
Conclusion: All *Salmonella* spp. isolates tested were susceptible to CIP. Resistance to NAL was found in 16% of isolates and was more frequent among *S. enteritidis* strains. Twenty-eight (14.7%) isolates showed decreased susceptibility to CIP. Determination of NAL resistance by the disk diffusion method was effective for the detection of decreased susceptibility to CIP. The method can be employed by diagnostic laboratories as a screening test.

P1437 Effect of quinolones against slowly growing bacteria

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Background: Bacteria growing in vivo multiply much more slowly than in vitro. The average generation time (*g*) of *E. coli* grown in vitro in kidney homogenate was 0.6 h as compared with 00.35 h for the broth-grown counterpart; the same strain causing pyelonephritis in an in vivo infection model has a generation time of 3.2 h. Therefore, the effect of different generation times on the bactericidal activity of quinolones was studied in batch cultures.
Methods: By limiting the nutrient supply, generation times were lengthened from approximately 0.45–3.9 h. Alternatively, the quinolones were added to the bacterial cultures during the lag-, exponential- and stationary phase. Recent clinical isolates of *E. coli*, *S. epidermidis*, and *S. aureus* (three strains each) were exposed to multiples of the MICs of ciprofloxacin, levofloxacin, or norfloxacin. The killing rates were calculated in analogy to the growth rate. Bacteria were grown in brain heart infusion broth in ambient air at 37°C.
Results: The mean killing rates of the three quinolones against the rapidly growing *E. coli* and *S. aureus* and their slowly growing counterparts is summarized in the table (analogous data were obtained for *S. epidermidis*):

	E. coli		*S. aureus*	
	$g = 0.38$ h	$g = 3.72$ h	$g = 0.40$ h	$g = 3.87$ h
CIP	65.9	59.1	2.7	3.2
LEV	21.5	12.8	0.8	1.1
NOR	16.5	9.4	1.6	2.1

Conclusions: CIP is the most bactericidal agent amongst the quinolones tested. Slow growth of *E. coli* affects the activity of CIP minimally, but that of LEV and NOR significantly ($P < 0.001$). In contrast, the activity of the quinolones is minimally increased by slow growth of *S. aureus*.

P1438 Activity of quinolones against attached *Staphylococcus epidermidis*: individual resistance phenotypes

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Staphylococcus epidermidis is a common pathogen in chronic, medical device-associated infections, which is able to attach on polymeric surfaces and to develop multilayered biofilms. Attached *S. epidermidis* displays reduced susceptibly against antimicrobial substances and little correlations between standard susceptibility tests and clinical outcome of antibiotic treatment were observed. In this study we investigated the effect of ciprofloxacin and the group IV quinolones gatifloxacin, gemifloxacin, and moxifloxacin on three biofilm-positive wild type strains and their isogenic biofilm-negative mutants. in the recently developed minimal attachment killing (MAK) assay. Depending on strain and investigated quinolone a heterogeneous MAK (MAKhetero) could be distinguished from a homogeneous resistance (MAKhomo) which corresponds to the model of few persisters within attached cells under antibiotic treatment. For the biofilm-negative mutants a lower MAKhomo was observed as for the corresponding wild types for some of the tested quinolones. This seems to be a result of higher bacterial inocula of wild-type strains, whereas the MAKhetero concentrations were comparable for mutants and wild types for nearly all of the tested antibiotics and strains. These data indicate that biofilm formation is not necessary for persistence of attached *S. epidermidis* cells under antibiotic treatment with quinolones and could explain therapeutic failure in foreign body-associated infections due to biofilm-negative *S. epidermidis* isolates. However, due to the increase of cells within a biofilm similar inoculum effects might be of relevance for some quinolones as observed for planktonic cells. The individual resistance phenotypes of the investigated strains with different antibiotics suppose that the MAK detection could help to predict the outcome of therapy in foreign body-associated infections with both biofilm-positive and biofilm-negative *S. epidermidis*. Thereby the group IV quinolones displayed relatively high activity against individual attached staphylococcal isolates, indicating a possible option for the treatment of foreign body-associated infections due to these isolates with the respective quinolones.

P1439 Therapeutically aspects in urinary tract infections: the resistance pattern of Gram-negative bacilli to aminoglycosides, quinolones and other antibiotics

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Background: The objective of our study is to describe the resistance pattern of strains isolated from patients with urinary infections.
Methods: Antimicrobial susceptibility testing of isolated bacilli was determined by disk diffusion method as recommended by NCCLS, using a panel of 23 antibiotics: quinolones, aminoglycosides, nitrofurantoin, tetracycline, trimethroprim–sulfamethoxazole, fosfomycin, and chloramphenicol discs.
Results: The pathogens identified from urine culture were *Pseudomonas* sp., *E. coli*, *Klebsiella oxytoca*, *Proteus* sp., *Acinetobacter*, *Morganella morganii*. *Acinetobacter calloaceticus* strains were resistant to aminoglycosides, *P. aeruginosa* strains showed a high resistance to amikacin, netilmicin and a low resistance to gentamicin and tobramicin. *Klebsiella*, *Proteus*, *E. coli*, *P. fluorescens*, *Enterobacter*, *Morganella* strains did not show resistance to aminoglycosides. *Acinetobacter* strains were resistant to quinolones. All *P. aeruginosa* strains were resistant to pefloxacin, showing a high resistance to nalidixic acid, ciprofloxacin (75%), ofloxacin (50%) and a low resistance to levofloxacin (25%). *E. coli* strains showed a low level of resistance to pefloxacin (10%) but all were susceptible to netilmicin. *P. fluorescens*, *Enterobacter*, *Klebsiella*, *Proteus*, *Morganella* strains were susceptible to nalidixic acid, ciprofloxacin, pefloxacin, ofloxacin and levofloxacin. Resistance rates of strains to tetracycline were high for *Proteus mirabilis, A. calloaceticus* (100%), *P. aeruginosa* (75%), *E. coli* (52.4%). *Enterobacter* and *Morganella* were susceptible to tetracycline. The resistance to trimethoprim/sulfamethoxazole significantly rose from 0% for *Enterobacter* and *Morganella*, to 14.3% for *Klebsiella*, to 27.3% for *E. coli* and 75% for *P. aeruginosa*. Nitrofurantoin, is still active against *P. fluorescens*, *E. coli*, *Klebsiella, Enterobacter* but it is inactive on *Proteus*, *P. aeruginosa*, *Morganella* and Acinetobacter. To fosfomycin, 50% of *Enterobacter* and all of *Acinetobacter*, *Morganella* strains were resistant, but fosfomycin is effective against *Pseudomonas* sp., *E. coli*, *Klebsiella*.
Conclusions: There was significant increase in prevalence of antibiotic resistance among the most common uro-pathogens, which raises difficulties in treating urinary tract infections with currently used antibiotics. Aminoglycosides and quinolones are no longer of first choice in treatment of urinary tract infections.

P1440 Questioning the possible synergistic effect of gentamicin with three fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin) in subminimal inhibitory concentrations on virulence factors of uropathogenic *Escherichia coli*

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Objectives: Gentamicin (G) is one of the powerful antibacterial against Gram-negative bacteria, has a rapid bactericide effect, has a significant effect in subminimal inhibitory concentrations (sub-MICs), and is relatively cost-effective. But gentamicin has a narrow antibacterial spectrum and has many

dose-dependent side-effects. Fluoroquinolones [ofloxacin (O), ciprofloxacin (C), levofloxacin (L)] have broad antibacterial spectrum, have rapid antibacterial effects also in sub-MICs, but are not cost-effective.
Methods: MICs (microplate MIC determinations were done), hydrophobicity (3.2–0.2 M of salt aggregations were determined on bacterial surfaces in 1/2–1/32 × MICs), hemagglutination (slide agglutination tests were determined by naked eye in 1/2–1/32 × MICs), adhesion on uroepthelial cells (healthy and non smoker ladies' uroepithelial cells were pooled – bacteria were counted which adhered to 40 cells in each slide in 1/2–1/32 × MICs), motility (swarming zones after 24 h of incubation at 37C were determined in 1/2–1/32 × MICs), time-kill determinations (0–6–12–24 h of colony counts were done in MIC values) against three strains of *Escherichia coli* [ATCC 25922 HA + mannose resistant, and two clinical isolates: one is mannose resistant (HA + 27096), the other is mannose susceptible (HA-27843)] were realized.
Results: Results showed us a clear-cut decrease in MICs, and synergistic interactions in adhered bacteria numbers on uroeptihelial cells, in hydrophobicity, in motility especially in G + L combinations in sub-MICs (more detailed results will be presented in congress hopefully).

Table 1 MIC values of antibacterial in combinations and in their own.

MIC results (mg/L)	ATCC 25922	HA+27096	HA-27843
Ciprofloxacin (C)	0.031	0.062	0.031
Ofloxacin (O)	0.062	0.5	0.031
Levofloxacin (L)	0.125	0.031	0.125
Gentamicin (G)	1.0	1.0	0.5
G + C	0.062	0.031	0.062
G + O	0.125	0.125	0.031
G + I	0.25	0.5	0.25

Conclusion: In conclusion, this preliminary study may be promising for a combined therapy protocol in the favor of gentamicin when considered its dose-related side-effects during prolonged therapies in uropathogenic *Escherichia coli* related infections.

P1441 The influence of suprainhibitory concentrations of fluoroquinolones on some virulence factors of *Stenotrophomonas maltophilia*

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Objectives: To determine and compare the postantibiotic effect (PAE) of fluoroquinolones (ciprofloxacin, enoxacin, ofloxacin, pefloxacin) for two clinical *Stenotrophomonas maltophilia* strains as well as to evaluate the effect of this pharmacodynamic parameter on the surface hydrophobicity, motility and lipase production of both strains studied.
Methods: The PAE was induced by suprainhibitory 2× and 4 × MIC concentrations of the antibiotics for 0.5 h and the antibiotics were eliminated by dilution. The hydrophobicity was determined on the basis of bacterial adherence to hydrocarbon–xylene (BATH) and salt aggregation test with ammonium sulfate (SAT). The assay of motility was performed on the semisolid agar medium (0.35%). The lipase activity was determined spectrophotometrically in sterile culture filtrates after incubation with Tween 80 in Tris–HCl and $CaCl_2$ buffers.
Results: The PAE of all fluoroquinolones at 2 × MIC were on average 2.9 h longer for strain no. 5777 than for strain no. 27823. The duration of the PAE was concentration dependent. The longest PAEs were induced at 4 × MIC by pefloxacin for strain no. 27823 (6.2 h) and by both ciprofloxacin and ofloxacin for strain no. 5777 (7.2 and 8.7 h, respectively). The most expressive inhibition of strain no. 27823 adhesion after ciprofloxacin was found at both 2× and 4 × MIC to 46.5 and 30.5%, respectively. After pefloxacin at 4 × MIC adhesion was inhibited to 41.8%. Adhesion of strain no. 5777 was inhibited to 47.1% after 4 × MIC of ciprofloxacin. The lipase production by strain no. 5777 in the PA phase was moderately stimulated. Only ciprofloxacin at 4 × MIC suppressed its production to 23.2% against control. The effect of suprainhibitory concentrations of fluoroquinolones tested on the motility of both strains was very weak and was concentration-independent.
Conclusions: The results suggest that any consideration of postantibiotic effects should include the residual antibiotic effects on virulence factors, in addition to the defined suppression of bacterial re-growth.

P1442 Antibacterial effects of enoxolone on periodontopathogenic and capnophilic bacteria isolated from specimens of periodontitis patients

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Objectives: Enoxolone is a major component of a traditional plant called Licorice. This substance has several pharmaceutical properties including anti-inflammatory, antiviral and antifungal activities. Microbiological studies have identified more than seven periodontopathogen in periodontal pockets, which less than four species are capnophile. The purpose of this study was to investigate the in vitro antibacterial effects of enoxolone against isolated periodontopathogenic and capnophilic bacteria.
Methods: Total specimens were collected with sterile paper points from the deepest periodontal pockets of 400 patients. The specimens were cultured under capnophilic condition on selective media. Biochemical tests were used to identify isolated bacteria. Antibacterial activities of enoxolone against isolates were investigated by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods.
Results: Isolated bacteria were *Actinobacillus actinomycetemcomitans* (46.5%), *Eikenella corrodens* (30%) and *Capnocytophaga* species (34%). The MIC of enoxolone were 8, 16 and 8 mg/L, respectively, and the MBC was 16 mg/L for all species.
Conclusion: It is concluded that enoxolone with appropriate concentration is effective against isolated periodontopathogenic and capnophilic bacteria.

Epidemiology of resistance 5

P1443 Antimicrobial drug resistance patterns of human *Salmonella enterica* strains isolated during 2000–2001 in Greece

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Objectives: Increasingly in recent years, high rates of antimicrobial drug resistance are being observed in *S. enterica*, often due to transferable resistance genes and arising among serotypes previously considered rare. The aim of this study was to continue the monitoring of antimicrobial drug resistance among the most important *S. enterica* serotypes in Greece for the years 2000–2001.
Methods: The study sample consisted of 536 isolates representative of 13 serotypes: Abony (8), Blockley (53), Brandenburg (10), Bredeney (6), Enteritidis (253), Hadar (37), Heidelberg (11), Infantis (33), Kottbus (8), Montevideo (12), Newport (7), Typhimurium (87), Virchow (13). They were tested on Mueller-Hinton agar by a disc diffusion assay according to NCCLS recommendations to 16 antimicrobial agents: ampicillin (Am), cefotaxime (Ctx), ceftazidime (Caz), amoxicillin/clavulanic acid (Amc), kanamycin (K), amikacin (An), netilmicin (Net), tobramycin (Tm), gentamicin (Gm), streptomycin (S), chloramphenicol (C), tetracycline (Te), sulfonamides (Sss), trimethoprim (Tmp), nalidixic acid (Na), ciprofloxacin (Cip).
Results: All isolates were susceptible to ciprofloxacin and amikacin. No high level resistance was observed in *Salmonella* Abony, Bredeney, Heidelberg and Montevideo. Resistance rates to the other antibiotics in the remaining serotypes ranged from 0.2% (ceftazidime and cefotaxime) to 30% (tetracycline); resistance to nalidixic acid was at 18%.
Conclusions: While resistance to third generation cephalosporins was negligible and no resistance to ciprofloxacin was observed, rates of resistance to nalidixic acid were considerable, especially for Hadar (95%), Blockley (38%), and Enteritidis (13%). Nevertheless, in comparison to previous years, mono-resistance to ampicillin, pentaresistance to AmCSSssTe and SKCTeNa,

remained the dominant resistance phenotypes for Enteritidis (33%), Typhimurium (21%) and Blockley (30%), respectively.

P1444 Antibiotic susceptibility of *Listeria monocytogenes* in Denmark, 1958–2001

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Objectives: In order to see if susceptibility of Danish *L. monocytogenes* strains has changed during the years we examined a collection of human isolates from the period 1958–2001. We furthermore wanted to compare two methods of in vitro susceptibility testing for *L. monocytogenes*.
Methods: One hundred and six strains isolated predominantly from blood cultures and cerebrospinal fluids were examined together with three reference strains. Susceptibility to the following antibiotics was tested by the *E*-test method and by Oxoid discs using Iso-sensitest agar: penicillin G, ampicillin, meropenem, gentamicin, sulphamethoxazole, trimethoprim, ciprofloxacin, erythromycin, vancomycin, linezolid, chloramphenicol and tetracycline.
Results: Both methods in the main demonstrated sensitivity to all the antibiotics. Results differed between the two methods for sulphamethoxazole and ciprofloxacin: All strains were sensitive to sulphamethoxazole when examined by disc diffusion, but only 72% were sensitive using the *E*-test method. For ciprofloxacin, 96% were intermediate sensitive by disc diffusion, while 97% were sensitive by the *E*-test method.
Conclusion: The antibiotic susceptibility of *L. monocytogenes* has not changed in Denmark from 1958 to 2001. There is good agreement between results of *E*-test and Oxoid disc methods, except for sulphamethoxazole and ciprofloxacin.

P1445 Surveillance of linezolid resistance in Germany

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Objectives: Evaluation of susceptibility to linezolid of grampositive pathogens from hospitalized patients reported by a network of 86 clinical microbiology laboratories throughout Germany
Methods: Each laboratory was requested to provide routine susceptibility data of 100 consecutive strains (35 *S. aureus*, 30 coagulase-negative staphylococci, 20 enterococci, and 15 streptococci). Most participants used the disk diffusion test according to NCCLS or German DIN guidelines. As DIN breakpoints for linezolid do not yet exist, those approved by the European Regulatory Agency (EMEA) were applied. For quality control retesting of 10% of all strains as well as strains classified as resistant to linezolid is ongoing.
Results: The susceptibility results of routine testing comprised 7201 isolates: 3844 *Staphyococcus aureus*, 773 *Stapylococcus epidermidis*, 1188 *Enterococcus fecalis*, 524 *Enterococcus fecium*, 480 *Streptococcus pneumoniae*, and 392 *Streptococcus pyogenes*. Most specimens were recovered from skin (29.4%), blood (23.4%), urine (9.6%) and respiratory tract (8%). Most frequent infection sites were SSTI (30%), upper and lower RTI (19.1%), foreign body/catheter-infections (10.5%) and UTI (9.8%). In 18.5% of patients the site of infection was unknown. Oxacillin resistance was observed in 17.1 and 58.7% of *S. aureus* and *S. epidermidis*, respectively. Among the isolates of *S. aureus* and *S. epidermidis* 99.4 and 99.7% were classified as susceptible to linezolid. In *E. fecalis* 96.2 and 99.4% were reported as susceptible to ampicillin and vancomycin, respectively. Against linezolid 96.9% of strains were classified as susceptible and 2.9% as resistant. In *E. fecium* ampicillin resistance was very common (83.9%), but vancomycin resistance was low (4.5%). The proportions of linezolid susceptible/resistant isolates were 93.8%/2.3%. Linezolid resistant streptococci were not reported. Of those isolates, that were reported as 'resistant' to linezolid in routine susceptibility testing and so far re-tested in a reference laboratory exhibited MICs of 4 mg/L or below, i.e. these isolates were not resistant to linezolid applying both EMEA and NCCLS interpretive criteria.
Conclusions: Based on routine susceptibility testing the prevalence of linezolid resistance in staphylococci and streptococci is extremely low in Germany. Isolates showing linezolid resistance in routine susceptibility testing should be re-evaluated.

P1446 Antibioresistance and other virulence aspects in *Aeromonas* strains isolated in aquatic environments in Danube Delta, Romania

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Introduction: In the last 30 years it was pointed out the evident implication of aquatic *Aeromonas* species in human pathology and the high mortality in extraintestinal *Aeromonas* infections due to poor knowledge of their antibioresistance patterns.
Purpose: To investigate the relationship between the virulence and antibiotic resistance determinants in 115 *Aeromonas* strains isolated in 2001 from different aquatic sources (salmastre water, aquatic plants, frogs, fish enteric content, fish sapling, snake and oyster shells), as well as their temperature dependence (4°C, 28°C, 37°C), in order to evaluate the contribution of aeromonades to the aquatic reservoirs of virulence and resistance.
Methods: Fourteen enzymatic tests (including ELISA and NAD-degradation for detecting CT-related toxins); diffusion method (NCCLS 2000) and by phenotypic tests for beta-lactamases.
Results: The *Aeromonas* strains did not cultivate at 4°C in the presence of antibiotics, while at 28°C and 37°C they exhibited similar antiobioresistance patterns: 96.6% exhibited constitutive resistance to penicillins (PEN-R, AMX-R) by penicillinase production, out of which 45% produced beta-lactamases sensitive to inhibitors (AMX-R, AMC-S) and 55% beta-lactamases resistant to inhibitors (AMX-R, AMC-R); 26% were FOX -R, 5.1% were carbapenemase producers (IMP-R) and a low number of *Aeromonas* strains exhibited multiple resistance markers (NOR-R, TCY-R, CHL-R, SXT-R) indicating the possibility of an efflux mechanism. Amylase, caseinase and lipase tests exhibited constant high positivity undependable of the incubation temperature; lecithinase at 4°C; slime, DNA-se, gelatinase at 28°C, esculin hydrolysis at 28°C and 37°C and finally Kanagawa hemolysin at 37°C; exception made the strains isolated from fish enteral content exhibiting constantly low levels for the most of the tested factors at 37°C. Two *Aeromonas hydrophila* group I produced a cholera-like toxin revealed by ELISA specific for CT, while 68.5% of the tested strains produced another ADP-rybosilating toxic factor revealed by NAD-degradation reaction.
Conclusion: The expression of the virulence factors had not significantly differed with the source of isolation, but with the incubation temperature, this aspect pleading for their specific role in strains survival, resistance and pathogenicity. The high levels of antibioresistance and other virulence features in aquatic *Aeromonas* strains are pleading for their implication in human pathology.

P1447 Antibiotic resistance trends in Canadian strains of *Streptococcus pneumoniae*

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Objectives: To determine antibiotic resistance trends in Canadian strains of *S. pneumoniae* (SP).
Methods: In 1988 and from 1993–June 2002, 16 787 SP isolates from 192 labs were submitted for susceptibility testing according to NCCLS protocols. Data on outpatient antibiotic use was obtained from IMS Canada. We examined the trends in antibiotic use and resistance rates in SP in Canada.
Results: Of the 16 787 SP isolates, 6350 were from blood/CSF, 5709 were respiratory specimens, and 4728 from other sites. The overall use of

	1988	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Pen NS	2.5	5.7	8.1	8.8	12.2	13.4	14.8	13.6	12.6	14.5	15.1
Pen R	0	0.9	1.3	2.2	4.1	6.5	5.6	5.9	5.9	6.8	6.7
Eryth R	1.2	1.9	3.4	3.1	5.1	6.8	10.5	10.0	11.2	12.9	13.1
Clinda R	1.2	0	1.7	1.3	2.4	3.6	5.1	4.9	5.6	5.9	6.1
Tmp/Smx R	3.7	3.8	4.6	9.6	12.6	14.7	12.0	12.0	11.4	11.9	13.3
Tet R	2.5	1.4	2.3	3.4	2.4	6.3	9.0	7.9	8.1	9.2	9.2
Cipro (≥4 mg/L)	0	0.5	0.8	0.7	0.8	1.8	1.8	1.6	1.4	2.4	2.3

macrolides have stabilized while fluoroquinolone use continues to rise in Canada.
Conclusions: Despite decreasing outpatient use of penicillin, TMP/SMX, and tetracyclin, there is no decrease in resistance to these agents observed. Increasing fluoroquinolone use is associated with increasing ciprofloxacin resistance which rose in 1996/7 and again in 2000/1 ($P = 0.03$) but has remained unchanged in the first 6 months of 2002.

P1448 Prevalence of mupirocin resistance in isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* in three Central European countries: results of the Antimicrobial Resistance Surveillance Study of the Paul-Ehrlich-Society for Chemotherapy, 2001

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Objectives: Mupirocin calcium ointment is a topical antibiotic indicated for the eradication of nasal carriage of staphylococci, including methicillin (oxacillin)-resistant strains. Resistance to mupirocin is related to alterations in the isoleucyl-tRNA synthetase. It is either of low-level (LL) due to mutations within the its gene or of high-level (HL) due to the acquisition of the *mupA* gene. The clinical significance of LL is debatable, whereas strains with HL cannot be eradicated with mupirocin. In this study we assessed the prevalence of resistance to mupirocin in isolates of *S. aureus* and *S. epidermidis* obtained from clinical specimens.
Methods: In November 2001, a total of 787 isolates of *S. aureus* and 456 isolates of *S. epidermidis* were collected from 26 clinical microbiology laboratories distributed throughout three Central European countries (Austria, Germany, and Switzerland). Minimal inhibitory concentrations of mupirocin were determined using the broth microdilution procedure according to the standard of the German DIN. The breakpoints used were 4 mg/L (susceptible), 8–256 mg/L (LL) and 512 mg/L (HL).
Results: Rates of LL- and HL-resistance were 2.9 and 0.9% in *S. aureus*, and 9.4 and 3.3% in *S. epidermidis*, respectively. Resistance to mupirocin was almost exclusively observed in oxacillin-resistant isolates. The majority of oxacillin-resistant isolates exhibited LL-resistance to mupirocin (table).

	Resistance (% of isolates)		
	LL	HL	Total
S. aureus			
Oxa-S ($n = 624$)	0.2	0.3	0.5
Oxa-R ($n = 163$)	15.6	3.5	19.1
S. epidermidis			
Oxa-S ($n = 142$)	0.9	0	0.9
Oxa-R ($n = 314$)	17.0	12.9	4.1

Conclusion: Resistance to mupirocin was more common in *S. epidermidis* than in *S. aureus*. Moreover, resistance to mupirocin was nearly always related to oxacillin-resistant isolates. Approximately 3 and 5% of oxacillin-resistant *S. aureus* and *S. epidermidis*, respectively, exhibited HL-resistance to mupirocin. We strongly recommend a judicious use of mupirocin in order to keep HL-resistance to mupirocin at a low level.

P1449 Retrospective review of the increase in MRSA bacteremia in one hospital over 10 years

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Objectives: To document the increase in MRSA bacteremia in one hospital from 1992 to 2001, and to analyze available data which might help explain the increase.
Methods: We analyzed diagnostic laboratory results for the Royal Infirmary of Edinburgh (RIE) and MRSA typing data from reference laboratories.
Results: The total number of episodes of *S. aureus* bacteremia (MSSA and MRSA) increased from 89 in 1992 to 257 in 2001. MRSA bacteremia was first observed in RIE in 1994, and increased in frequency to 148 episodes in 2001. The 1994 MRSA cases coincided with the appearance of EMRSA-15, and the increase over the next few years paralleled the rising prevalence of EMRSA-15, though not all of the early MRSA bacteremias were EMRSA-15. EMRSA-16 was first seen in 1997, and has since accounted for a large proportion of bacteremias. The ratio of bacteremia to other isolates was higher for MRSA (7.8%) than for MSSA (4.6%), despite extensive screening for MRSA. The early cases of MRSA bacteremia were associated with a few specialist clinical areas, and these areas have continued to account for most of the cases, but almost all specialities have had some cases. There was no obvious trend to older patients being affected over time. The increase in MRSA bacteremia could not be explained by other documented changes in health care practices.
Conclusion: The arrival and spread of EMRSA-15 and EMRSA-16 are the major documented factors associated with the increase in MRSA bacteraemia.

P1450 Telithromycin resistance among streptococci in Finland

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Objectives: Telithromycin is a new antibiotic belonging to the ketolide group. Here we present the results of the first year of a 5-year study of the in vitro activity of telithromycin against *Streptococcus pyogenes* and *Streptococcus pneumoniae* strains in Finland. Thus far all the *S. pneumoniae* strains tested have been susceptible to telithromycin, regardless of their macrolide resistance mechanisms. It will be interesting to follow the development of telithromycin resistance levels and mechanisms in *S. pyogenes* and *S. pneumoniae*, and relate this to the antibiotic usage.
Methods: Fifty consecutive clinical isolates of *S. pyogenes* and *S. pneumoniae* are collected each year from each of the 26 FiRe laboratories situated all over Finland. Strain collection started at the beginning of May 2002. Each strain is tested for susceptibility to various antibiotics using the agar-dilution technique. Macrolide-resistant genes [*mef(A)*, *erm(B)* and *erm(TR)*] are determined using PCR, and mutations causing macrolide resistance by sequencing. Antibiotic consumption data is obtained from the National Agency of Medicines.
Results: During the year 2002, 614 *S. pneumoniae* and 662 *S pyogenes* strains from 13 FiRe laboratories were collected. Of these, 132 *S. pneumoniae* and 106 *S. pyogenes* strains have been analyzed thus far. Of the *S. pneumoniae* strains, 19.6% (26/132) were resistant (MIC $\geq$1 mg/L) to erythromycin. The resistance mechanisms were the *mef(A/E)* and *erm(B)* genes and an unknown mechanism in 19, 5 and 4 strains, respectively. Telithromycin MICs of 1–4 mg/L were found in 10 strains. All these strains had either the *erm(B)* or *mef(A/E)* gene. Of the *S. pyogenes* strains, 12.2% (13/106) were erythromycin resistant (MIC $\geq$1 mg/L). The *erm(TR)* gene was the most common (11 strains). *Erm(B)* was found in two strains and *mef(A/E)* in one strain. One strain, constitutively expressing *erm(B)*, had a telithromycin MIC value of 64 mg/L.
Conclusion: Preliminary data indicates that macrolide resistance among *S. pneumoniae* and *S. pyogenes* clinical isolates is increasing in Finland. There are also a few *S. pneumoniae* strains with elevated telithromycin MICs. Unlike most of the *S. pneumoniae* strains, *S. pyogenes* strains with a constitutively expressed *erm(B)* gene are resistant to telithromycin.

P1451 Prevalence of primary antibiotic resistance in *Helicobacter pylori* at two different locations in Belgium (1998–2002)

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Objectives: Antimicrobial resistance and patient non compliance are the two main factors associated with failure of *H. pylori* eradication. Our aim was to survey frequencies and levels of primary in vitro antibiotic resistance in two different geographical areas in Belgium, and to investigate possible links with disease severity, gender, age, and ethnicity of patients.
Methods: Gastric antral and body biopsies for *H. pylori* cultures were received from two different endoscopy departments: Saint-Luc University hospital (Brussels), 259 isolates (2000–2002) and Mont-Godinne University hospital

(Yvoir, South Belgium), 345 isolates (1998–2002). Specimens were cultured and isolates were tested by disc diffusion and/or *E*-test for susceptibility to metronidazole (Mtz), clarithromycin (Cla), amoxicillin (Amx) and tetracycline (Tet).
Results: The overall prevalence of *H. pylori* infection in the attending population was 27% and did not vary significantly over years or between the two locations. Global resistance rates to Mtz and Cla averaged 31 and 18%, respectively. Less than 1% of the isolates were resistant to Tet while no resistance to Amx was found. Mtz resistance varied markedly between population groups (43% in non Belgian-born vs. 26% in Belgian-born individuals) and between genders (38% in females vs. 26% in males). Breakdown of resistance by location also showed marked geographical resistance for Mtz resistance (35% in Brussels vs. 27% in Yvoir) and Cla resistance (13% in Brussels vs. 22% in Yvoir). The higher proportion of non-Belgian born individuals in Brussels (33% of all patients vs. 9% in Yvoir) mainly accounted for the higher Mtz resistance rates at the former location. No significant differences in Mtz nor in Cla resistance were observed between ulcer- or nonulcer dyspepsia-associated *H. pylori* isolates.
Conclusion: *H. pylori* Mtz and Cla resistance did vary markedly between the two different locations with non-Belgian birth being a key risk factor for infection with a primary Mtz resistance strain. The higher rate of Cla resistance in Yvoir could possibly reflect a higher consumption of macrolides in this local population. Overall our results highlight the importance of relying on local resistance figures in order to adapt and optimize the choice of the anti-*H. pylori* treatment strategies.

P1452 The prevalence of ACSSuT-R type *Salmonella enterica* serotype Typhimurium in Turkey

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Objectives: Strains of *Salmonella enterica* serotype Typhimurium phage type DT104 have become a world-wide health problem. Isolates of this phage type often possess resistance of ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclin (ACSSuT). The aim of this study was to determine prevalence of ACSSuT resistance pattern among human isolates of *S. Typhimurium* in Turkey, and to investigate prevalence of decreased susceptibility to ciprofloxacin (MIC, 0.125–1 mg/lt) in ACSSuT R-type *S. Typhimurium* strains.
Methods: A total of 620 Salmonella isolates from humans (296 Enteritidis, 215 Typhimurium, 37 Paratyphi B, 18 Typhi, 1 Paratyphi A, 53 Seogroup C) were collected from 10 cities in Turkey, between July 2000-June 2002. Antimicrobial susceptibilities (ampicillin:A, cefotaxime:Cf, gentamicin:G, chloramphenicol:C, tetracyclin:T, trimethoprim:Tm, ciprofloxacin:Cp, streptomycin:S, sulphonamides:Su) were determined by agar dilution tests following the NCCLS guidelines and intermediately resistant strains were included in the resistant category.
Results: Thirty-two (14.88%) *S. Typhimurium* isolates were susceptible to all antimicrobials. Eleven (5.11%) isolates were resistant to a single antimicrobial. The most prevalent resistance pattern was ACSSuT (156, 72.55%). Other resistance patterns were consecutively AT (5), ACSSuTTm (4), ACST (2), ACGSSuTTm (2), ACfCSuTTm (2), AC (1) and ACT (1). Decreased susceptibility to ciprofloxacin was observed in 6.97% (15) of all *S. Typhimurium* strains and in 5.12% (8) of the strains with ACSSuT resistance pattern.
Conclusions: These results indicated that ACSSuT R-type *S. Typhimurium* strains were common in Turkey and decreased susceptibility to ciprofloxacin in multiresistant *S. Typhimurium* strains was a newly encountered problem.

P1453 High prevalence of ESBL producing multiresistant *E. coli* from UTI infections in a university hospital in Egypt

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Objectives: To determine the antibiotic susceptibilities and the prevalence of ESBL in clinical *E. coli* strains from urinary tract infections isolated in Egypt.
Methods: During the period June 2001 to September 2001 103 isolates were collected from urinary tract infections at a university hospital in Cairo, with 46 strains being *E. coli*. These isolates were further characterized by determining MIC values to 33 different antibiotics according to NCCLS recommendations. Beta-lactamases were phenotypically differentiated by their MIC profiles to 7 beta-lactams and beta-lactam + beta-lactamase-inhibitor combinations. For ESBL producers plasmid profiles were determined and conjugation experiments were carried out.
Results: The overall resistance of the *E. coli* isolates was extremely high with a ciprofloxacin resistance of 41.3%, a resistance to aminoglycosides of 69.6%, a doxycycline resistance of 89.1%, and a resistance to trimethoprim/sulfamethoxazol of 97.8% according to NCCLS breakpoints. Only four of the strains (8.7%) were sensitive to ampicillin, hence producing no other beta-lactamase than the chromosomally encoded AmpC enzyme in a very small amount. Fourteen strains (30.4%) were phenotypically classified as harboring original spectrum beta-lactamases. The majority of strains (60.9%) produced extended spectrum beta-lactamases. These strains exhibited a pronounced multiresistance profile with most strains being resistant to four or five different classes of antibiotics. The analysis of plasmid profiles excluded the possibility of the spread of one single ESBL producing clone as eight different profiles were found. However, this finding also points to an intrahospital spread of plasmids and/or strains. In conjugations experiments plasmids conjugated with high efficiency.
Conclusion: In this study alarmingly high rates of ESBL producing multiresistant *E. coli* strains were found. Therapeutic options for these strains are restricted. Full susceptibility was only seen for aminopenicillins + beta-lactamase inhibitors, carbapenems and amikacin. There is an urgent need for appropriate antibacterial management and infection control.

P1454 Stability of fluoroquinolone resistance in Canadian *Streptococcus pneumoniae* isolates

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Objectives: The surveillance of fluoroquinolone resistance rates is essential as the respiratory fluoroquinolones, gatifloxacin, levofloxacin, and moxifloxacin, are increasingly used in the empiric treatment of *S. pneumoniae* infections. The aim of this study was to evaluate fluoroquinolone nonsusceptibility rates for *S. pneumoniae* isolated in Canada from 1997 to 2002.
Methods: Clinically significant isolates of *S. pneumoniae* were collected between 1997 and 2002 from 25 medical centers in 9 of the 10 Canadian provinces as a component of an ongoing national surveillance study and tested for their susceptibility to ciprofloxacin, gatifloxacin, levofloxacin, and moxifloxacin (NCCLS; M7-A5).
Results: The fluoroquinolone nonsusceptibility (*) rates for *S. pneumoniae* isolated in Canada between 1997 and 2002 are summarized in the table below.

	Ciprofloxacin		Gatifloxacin		Levofloxacin		Moxifloxacin	
Year	%	*n*	%	*n*	%	*n*	%	*n*
1997–98	1.38	1179	ND	0	0.42	1179	0	674
1998–99	1.42	1334	ND	0	0.06	1334	0.40	1278
1999–2000	1.63	1593	0.83	1559	1.13	1593	0.96	1559
2000–01	1.76	1421	0.91	1421	0.77	1421	0.77	1421
20001–02	1.88	1276	1.28	1254	1.49	1276	1.18	1276
Overall (1997–2002)	1.61	6803	0.94	4234	0.90	6803	0.74	6208

*Non-susceptibility was defined as $\geq$4 μg/mL, >1 μg/mL, >2 μg/mL, and >1 μg/mL for ciprofloxacin, gatifloxacin, levofloxacin, and moxifloxacin, respectively; ND, no data.

Conclusion: Fluoroquinolone nonsusceptibility rates for *S. pneumoniae* remain stable in Canada. Overall (1997–2002) nonsusceptibility rates are below 2% for ciprofloxacin and below 1% for gatifloxacin, levofloxacin, and moxifloxacin.

P1455 In vitro activity of telithromycin against *Streptococcus pneumoniae* isolated from respiratory tract infections in France in 2002

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Objectives: the objective of this study was to evaluate the in vitro activity of telithromycin (TEL) and other antibiotics commonly used in France against *Streptococcus pneumoniae* (SP) isolates responsible for respiratory tract infections in adult patients.

Methods: Four hundred and eighty-four clinical strains of SP were isolated from adult respiratory tract infections from February to April 2002 in 42 metropolitan French hospitals. MICs of TEL, penicillin (P), and erythromycin (E) were determined by microdilution method in a central laboratory. Susceptibility rates were calculated according to the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie. Determination of resistance mechanisms to E (ermB and mefA) was performed by PCR.
Results: The mean age of the patients was 58.1 ± 18.6 years and 68.6% of them were male. The diagnosis associated with the isolated strains were: pneumonia 58.7%, acute exacerbation of chronic bronchitis 18.4%, sinusitis 4.3%, others 18.6%. One hundred and forty-nine strains were isolated from at least one blood culture. From a total of 484 strains, 44.8% were susceptible (PS), 19% intermediate (PI) and 36.2% resistant (PR) to P; 49.4% were susceptible (ES) and 50.6% intermediate or resistant (EIR) to E. All but two of the EIR strains carried the *ermB* gene. The two remaining strains carried the *mefA* gene. The percentages of susceptibility to TEL and the MIC50/90 (mg/L) were the following:
All strains ($n = 484$): 98.6% S – 0.015/0.125
PS strains ($n = 217$): 100% S – 0.015/0.015
PI strains ($n = 92$): 97.8% S – 0.015/0.12
PR strains ($n = 175$): 97.1% S – 0.03/0.25
ES strains ($n = 239$): 100% S – 0.015/0.015
EIR strains ($n = 245$): 97.1% S – 0.03/0.25
There was no resistant strain to TEL.
Conclusions: Telithromycin demonstrates high in vitro activity against SP isolated from adult respiratory tract infections regardless the susceptibility to penicillin or erythromycin.

P1456 In vitro activity of levofloxacin against *Streptococcus pneumoniae*: results of the first study period in 2002

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Objective: The objective of this study was to evaluate the in vitro activity of levofloxacin (LVX) against *Streptococcus pneumoniae* strains isolated from respiratory tract infections in adult patients, collected from 42 French hospitals between February and April 2002.
Methods: The MICs of LVX, penicillin (PEN), and erythromycin (ERY) were determined by the microdilution method in a central laboratory. Susceptibility rates were calculated according to the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie. Quality control was performed with *S. pneumoniae* ATCC 49619 (wild-type) strain.
Results: The MICs 50/90 (mg/L) –% S/I/R were the following:
Overall strains ($n = 484$: LVX 1/1–99.6/0/0.4; PEN 0.12/2–44.8/19/36.2; ERY 2/>16–49.4/2.9/47.7
PEN-susceptible strains ($n = 217$): LVX 1/1–100/0/0; PEN 0.03/0.06–100/0/0; ERY 0.03/16–87.6/1.4/11
PEN-intermediate strains ($n = 92$): LVX 1/1–100/0/0; PEN 0.5/1–0/100/0; ERY >16/>16–34.8/4.4/60.8
PEN-resistant strains ($n = 175$): LVX 1/1–98.9/0/1.1; PEN 2/4–0/0/100; ERY >16/>16–9.7/4/86.3
The majority of isolates was recovered from lower respiratory specimens (71.9%) and/or blood cultures (30.8%) and was obtained from patients hospitalized in medical (35.9%) or intensive care units (34.3%). The most frequent diagnosis were pneumonia (58.7%) and acute exacerbation of chronic bronchitis (18.4%). Levofloxacin had a good activity against *S. pneumoniae* with 99.6% of susceptible strains. Resistance to penicillin and erythromycin was high, respectively, 36.2 and 47.7%.
Conclusion: The results of this in vitro survey shows the good activity of levofloxacin against *S. pneumoniae* whereas a high rate of resistance to penicillin and erythromycin is reported.

P1457 Patterns of organisms and their antimicrobial susceptibilities isolated from blood cultures in Larnaca General Hospital, Cyprus 1999–2002

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Objectives: To provide data on the patterns of organisms and their antimicrobial susceptibilities isolated from blood cultures in a district general hospital in Cyprus, where the availability of such data is lacking.
Methods: We performed a retrospective survey of all positive blood cultures received in the microbiology department of Larnaca General Hospital from 1999 to 2002. Isolates were identified using conventional microbiology methods and susceptibility tests were performed in accordance with NCCLS guidelines wherever possible. The clinical and laboratory data were entered into a database in WHONET 5 and the results analyzed in WHONET 5.
Results: From 1999 to 2002, 5260 blood culture bottles were received in the microbiology department, comprising 2416 sets. These resulted in 278 episodes yielding positive blood cultures. Of these, 105 (37.8%) were considered probable contaminants and 173 (62.2%) were considered clinically significant. Of the clinically significant isolates the commonest organisms were coagulase negative staphylococci 38/173 (22.0%), *Staphylococcus aureus* 34/173 (19.7%), *Pseudomonas aeruginosa* 17/173 (9.8%), *Klebsiella* spp. 16/173 (9.2%), *Escherichia coli* 11/173 (6.4%), *Acinetobacter* spp. 8/173 (4.6%), *Candida* spp. 7/173 (4.0%), *Enterobacter* spp. 7/173 (4.0%), *Enterococcus* spp. 7/173 (4.0%) and *Streptococcus pneumoniae* 5/173 (2.9%). Overall, 47.1% of *S. aureus* isolates were methicillin-resistant (MRSA) and the percentage of MRSA among *S. aureus* isolates increased from 33.3% in 1999 to 66.7% in 2002. No glycopeptide-resistant enterococci or penicillin-resistant *S. pneumoniae* were isolated. All *Candida* isolates occurred during 2001 and 2002. Quinolone resistance was higher among enterobacteriaceae than in *P. aeruginosa*. Carbapenem resistance in *P. aeruginosa* was 31.6% but was not detected in enterobacteriaceae or in *Acinetobacter* spp. Resistance to third generation cephalosporins was 38.1% among enterobacteriaceae and 50% in *Acinetobacter* spp.
Conclusions: MRSA and *Candida* spp. have emerged as important causes of bloodstream infection in our hospital. Surveillance of antimicrobial resistance is essential to optimize the empirical antimicrobial treatment of infection. It is essential that resources are provided so that such surveys may be carried out in Cyprus.

P1458 Multicenter study on antibiotic resistance of *Staphylococcus aureus* and the analysis of the clonal structure of MRSA in Poland

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Objective: To evaluate the antimicrobial susceptibility of *Staphylococcus aureus* isolated from 267 Polish medical centers and to reveal the clonal structure of the MRSA population.
Methods: A total of 1363 *S aureus* isolates were collected between 1.10.1999 and 15.11.1999 from 276 Polish medical centers. They represented five consecutive isolates per center (one isolate/one patient) and were recovered from various infection sites. Identification of methicillin resistance was performed by the screening technique and confirmed by the PCR detection of the *mecA* gene. MICs were determined by the agar dilution method according to NCCLS. Nineteen antimicrobials were tested for MRSA isolates and 24 for MSSA. MRSA isolates were typed by PFGE.
Results: Of the 1363 isolates 181 (13%) were methicillin-resistant. All the isolates were fully susceptible to vancomycin and teicoplanin. MRSA isolates were usually multidrug resistant. Only 18% of them were susceptible to tetracycline, 25% to gentamicin, 31% to erythromycin, and 42% to ciprofloxacin. Almost 23% of the isolates exhibited resistance to seven different antimicrobials. Out of MSSA isolates 79% were resistant to penicillin, 38% to tetracycline, and 9% to erythromycin. Only few isolates were resistant to mupirocin. The vast majority of the MRSA isolates represented five different epidemic clones that had been identified among Polish MRSA before. Eight new PFGE patterns have been observed however, they have been characterized sporadic isolates from single centers.

Conclusion: The representation of *S. aureus* isolates from more than 250 centers gave us the unique opportunity to study antimicrobial susceptibility of this organism in Poland. Less than 15% of the isolates turned out to be methicillin-resistant, which indicated that the MRSA frequency has most probably decreased in recent years. This may be attributed to improved diagnostic skills and implementation of infection control policy in more and more medical institutions in the country. Data presenting in this study revealed the increasing variety of clonal structure of MRSA strains in Poland.

P1459 The Libra Initiative: surveillance of the antibiotic susceptibility of urinary-tract infection pathogens in Europe from outpatients and inpatients during 1999/2000

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Objectives: To ascertain the prevalence of various uropathogens in outpatient (OUT) vs. in-patient (IN) urinary-tract infections (UTIs) and determine the susceptibility of these pathogens to relevant antimicrobials.
Methods: In total 3144 OUT UTI and 2608 IN UTI isolates were collected from 33 centers within 14 European countries (Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, the Netherlands, Portugal, Spain, Switzerland, Sweden and the United Kingdom), transported to a central laboratory and re-identified. Antibiotic susceptibility was determined using the NCCLS agar dilution method.
Results: Seventy-four bacterial species were identified causing UTIs. Of these, *Escherichia coli* was found to be the most prevalent pathogen in both OUT and IN (61.3 and 55.4%, respectively), followed by *Enterococcus fecalis* (8.3 and 8.6%), *Proteus mirabilis* (7.2 and 6.2%), *Klebsiella pneumoniae* (5.4 and 6.1%) and *Pseudomonas aeruginosa* (3.0 and 5.9%). The percentage susceptibility of *E. coli* in Europe as a whole for orally available antibiotics was ciprofloxacin (CIP; 93.3% OUT, 90.9% IN), nalidixic acid (87.4, 83.5%), amoxycillin (55.5, 52.0%), amoxycillin-clavulanate (82.4, 79.4%), cephradine (55.2, 50.6%), nitrofurantoin (NFT; 94.0, 91.7%), trimethoprim (74.4, 71.1%) and tetracycline (69.8, 66.4%). Therefore, CIP and NFT were the most active of these antibiotics against *E. coli*. However, nitrofurantoin is inactive against *P. aeruginosa* and only 0.4 and 0% of P. mirabilis from OUT and IN were found to be susceptible to NFT. For CIP, 69.9 and 58.1% susceptibility occurred for OUT and IN *P. aeruginosa* and 85.8 and 85.7% for OUT and IN *P. mirabilis*. For antibiotics available for parenteral administration only, *E. coli* OUT and IN susceptibilities were: ceftazidime (99.5, 98.6%), imipenem (100, 99.9%) and gentamicin (97.5, 97.2%).
Conclusions: CIP offered the best overall in vitro activity for an oral antibiotic and CIP activity was good compared with parenteral antibiotics, which is important because CIP is available in parenteral as well as oral form.

P1460 Identification of two widely disseminated strains of *Enterococcus faecalis* highly resistant to gentamicin and ciprofloxacin causing bacteremias in the United Kingdom

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Objectives: To investigate the basis of a significant association observed between high-level resistance to ciprofloxacin (Cp) and gentamicin (Gm) in isolates of *Enterococcus faecalis* collected in the United Kingdom & Ireland as part of the BSAC Bacteraemia Resistance Surveillance Program, 2001.
Methods: In 2001, 24 laboratories collected 152 consecutive isolates of *E. faecalis* from bacteremias; all were from separate patients. MICs to a panel of antibiotics were determined centrally, and identification was confirmed. Pulsed-field gel electrophoresis (PFGE) of SmaI-digested genomic DNA was performed to investigate the relatedness of selected isolates, and banding patterns were analyzed using BioNumerics software. The criterion of >80% similarity was used to define strains.
Results: Sixty of 66 *E. faecalis* isolates with high-level Gm-R (MICs $\geq$512 mg/L) were resistant to Cp (MICs $\geq$32 mg/L), compared with only 7 of 86 *E. faecalis* isolates with normal Gm susceptibility ($P < 0.0001$). This association was not seen for other *Enterococcus* spp. collected during the survey. To investigate this further, 38 isolates of *E. faecalis* highly resistant to Gm (MICs > 2048 mg/L) and Cp (MICs > 64 mg/L), and from 18 different hospitals, were compared by PFGE. Two large clusters were apparent after analysis of banding patterns; cluster 1 contained 14 isolates from seven hospitals that were related at $\geq$85% similarity; cluster 2 contained 10 isolates from 6 hospitals also related at $\geq$85% similarity. Clusters 1 and 2 were not closely related to each other (60% similarity). Isolates in both clusters were also resistant to erythromycin (MICs > 256 mg/L). Eighteen of 21 comparator isolates of *E. faecalis* from the same bacteremia survey (6 high-level Gm-R, Cp-S; 7 G-'S', Cp-R; 8 G-'S', Cp-S) did not fall into either of these clusters. Three comparator isolates grouped with cluster 2; two were from one hospital (both Gm MIC 256 mg/L, Cp MIC 128 mg/L); the third (Gm MIC 16 mg/L, Cp MIC 128 mg/L) was from the same hospital as several other group 2 isolates that had high-level Gm-R
Conclusions: The association between high-level Gm-R and Cp-R in *E. faecalis* from bacteraemia reflected the presence of multiple isolates of two widely disseminated strains. Dissociated resistance to Cp and Gm in some isolates of cluster 2 discounts direct genetic linkage of resistance genes. Further work is needed to characterize these 'epidemic' strains and to investigate the presence of virulence genes.

P1461 Comparison of serotyping and genotyping of Hungarian *Streptococcus pneumoniae* isolates

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Objectives: *Streptococcus pneumoniae* can cause a wide range of serious diseases with high morbidity and mortality. It is essential to determine the epidemiological and genetic relatedness of groups of bacteria in order to control diseases. Both phenotyping and genotyping methods are used for this purpose. In this study we have chosen pulsed-field gel electrophoresis (PFGE) for the molecular characterization of penicillin nonsusceptible Hungarian pneumococci and we compared the results to those obtained by serotyping as well as by antibiotic sensitivity.
Methods: Ninety-six *Streptococcus pneumoniae* strains were included in the study, all being penicillin nonsusceptible, isolated in Hungary in 2000–2002. Their identity confirmed by optochin sensitivity and the presence of the lytA gene. The susceptibility testing was performed according to the NCCLS guidelines including suitable control strains. Serotyping of the strains was done with the MAST typing antisera. PFGE was performed with the ApaI enzyme.
Results: The most prevalent serotypes were 6, 9, 14, 23 and 19. We found a significant correlation between macrolide sensitivity and strain serotype, but no clear correlation was seen with penicillin intermediate strains. Although 20 PFGE clones were found in all, 69% of the bacteria were in just four clones. Serogroup 9 showed the least genetic variation whereas serogroup 6 showed the greatest diversity. Different genetic clones harboured similar phenotypic features, suggesting horizontal transfer, but, in contrast, there were differences within one clonal lineage (loss or gain of resistance, serotype switch). We have found PFGE pattern identity between our serogroup 9 isolates and others from different countries of the world. Similarly there was identity between the serogroup 23 isolates from the UK and Hungary, showing some intercountry dissemination.
Conclusion: In contrast to previous reports, the Hungarian penicillin nonsusceptible pneumococcal population is very heterogeneous. The successful 9 V and 23F international serotypes appear to have reached Hungary. We have shown considerable diversity within individual serotypes. Indistinguishable serotype is not indicative of identical or even closely related clones. Additionally, serotypes do not always show a close correlation with resistance pattern, therefore serotyping alone is insufficient for epidemiological studies and the use of molecular typing is essential.

P1462 Quinolone resistance in clinical Enterobacteriaceae strains from urinary tract infections

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Objectives: To study the frequency and antimicrobial susceptibility of quinolone – resistant *Enterobacteriaceae* strains isolated from patients with urinary tract infections in our hospital.
Methods: We studied retrospectively the antibiotic susceptibility of 1693 *Enterobacteriaceae* strains isolated over a 3-year period (2000–2002) from patients with urinary tract infections. Isolation and identification of the

microorganisms was performed by conventional methods. The sensitivity to antimicrobial agents was tested by the disc-diffusion method (NCCLS recommendations).
Results: Of the strains tested 1415 (83.6%) were *Escherichia coli*, 167 (9.9%) *Klebsiella pneumoniae* and 111 (6.5%) *Proteus mirabilis*. The resistance rates to ciprofloxacin, ofloxacin, pefloxacin, norfloxacin and nalidixic-acid, respectively, was: *E. coli* (7.7–7.6–7.8–7.6–11.8%), *K. pneumoniae* (15.2–12.6–13.4–12.6–30.1%), *P. mirabilis* (14.4–14.3–15.5–13.6–29.4%). Of the cipR-*E. coli* strains 95.5% were also resistant to norfloxacin and pefloxacin whereas 99% of all norR-*E. coli* strains were also resistant to ciprofloxacin. All cipR and norR *K. pneumoniae* strains were resistant to the other four quinolones and 93.3% of the cipR-P. mirabilis strains were resistant to norfloxacin. Resistace rates to the other antibiotics for the cipR and norR-*E. coli* strains were, respectively: ampicillin 82.8–83.3%, trimethoprim/sulfamethoxazole 82.6%–85.2%, gentamicin 27.0–28.1%, cephalothin 60.8–61.2%, cefuroxime 29.4–30.6%, ceftazidime 11.8–12.2%, amoxicillin/clavulanic acid 24.5–25.5%. For *K. Pneumoniae*: ampicillin 100.0%–100.0%, trimethoprim/sulfamethoxazole 86.7–86.7%, gentamicin 43.8–43.8%, cephalothin 87.5–87.5%, cefuroxime 75.0–75.0%, ceftazidime 81.2–81.2%, amoxicillin/clavulanic acid 56.2–56.2%. For *P. mirabilis*: ampicillin 88.9–88.9%, trimethoprim/sulfamethoxazole 100.0–92.3%, gentamicin 33.3–33.3%, cephalothin 86.7–93.3%, cefuroxime 86.7–93.3%, ceftazidime 80.0–80.0%, amoxicillin/clavulanic acid 86.7–86.7%.
Conclusions: During the study period we observed a significant increase in the incidence of *Enterobacteriaceae* strains resistant to quinolones. The increase use of quinolones has resulted in the emergence of quinolone-resistant stains in urinary tract infections. We also observed high resistance rates to major classes of antimicrobial agents (80–100%) for the quinolone-resistant strains.

P1463 Clonal spread of methicillin-resistant *Staphylococcus aureus* at a tertiary care hospital

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Objective: To investigate the clonal relationships of methicillin-susceptible and methicillin-resistant *S. aureus* recovered from in and out patients.
Methods: Molecular epidemiology of 75 MRSA and 50 MSSA recovered from clinical specimens was studied by AP-PCR using M13 primer. Sixty-six of the MRSA and 32 of the MSSA were from hospitalized patients while the remaining (9 MRSA and 18 MSSA) were recovered from outpatients.
Results: AP-PCR analysis of MSSA and MRSA isolates produced 19 and 5 patterns, respectively. Among MRSA isolates, a group designated as pattern A contained 59 isolates, whereas patterns D and B, both of which differed from pattern A in only one band, contained 13 and one isolates, respectively. Eight (89%) of the isolates that were recovered from out-patients had molecular pattern A, and one (11%) pattern D.
Conclusions: Our results suggest that clonally related MRSA isolates have disseminated both in community and hospital setting. On the other hand, clonal spread is limited among MSSA.

P1464 Antibiotic susceptibility patterns and molecular epidemiology of *Streptococcus pneumoniae* in Izmir, Turkey

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Objective: To investigate the antibiotic susceptibility and clonal relationship of *Streptococcus pneumoniae* strains isolated at a tertiary care hospital located in Izmir, Turkey.
Methods: A total of 71 *S. pneumoniae* strains isolated from various clinical specimens (respiratory system, eye, blood and cerebrospinal fluid) between February 2001 and December 2002, were taken into the study. Penicillin (Pen) and erythromycin MICs were determined by *E*-test (AB Biodisk, Sweden) and by microdilution, respectively. Susceptibility patterns for other agents (clindamycin, tetracycline, chloramphenicol, cotrimoxazole, ciprofloxacin and levofloxacin) were investigated by disk diffusion. Clonal relationship among the isolates themselves and the similarities with 16 penicillin-resistant clones defined by the Pneumococcal Molecular Epidemiology Network (PMEN) were investigated by BOX-PCR using BOX-A1R primer. Isolates that had only one band difference were classified as clonally related.
Results: Three (4.2%) of the pneumococci were found to be high-level PenR; whereas 22 (30.9%) were low-level resistant and 46 (64.8%) isolates were susceptible to penicillin. Resistance rates of cotrimoxazole, tetracycline, erythromycin, clindamycin and chloramphenicol were found as 45, 33.8, 23.9, 23.9 and 12.6%, respectively. Quinolone resistance was not observed among the penicillin-susceptible and penicillin-resistant strains. Three, 19 and 37 unique BOX-PCR were identified patterns among PenR, PenI and PenS isolates, respectively. None of the Izmir isolates shared the same pattern with PMEN clones.
Conclusion: In spite of a relatively high ratio of penicillin resistance among pneumococci isolated in Izmir, significant clustering of the isolates was not detected by BOX-PCR and our isolates were different from the 16 widespread isolates over the world.

European surveillance of antibiotic consumption

P1465 Consumption of antibiotics in Austria: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, date accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Austria (8.1 million inhabitants in 2001), reimbursement data (100% social coverage) for the period 1997–2001 but only for Upper Austria, were provided by the Social Insurance Company of Upper Austria. AC data for the other regions and HC data are not available yet.
Results: In Upper Austria, AC use of antibiotics is characterized by a high use of broad-spectrum penicillins and a high use of macrolides.
Strengths and weaknesses: Data collection of AC data is ongoing. Reimbursement data will be provided by all regional Social Insurance Companies. Main problem of the Austrian data collection system remains the incomplete coverage of different classes of antibiotics. Antibiotics with a lower price than the prescription fee are not registered and will never be collected within the framework of the reimbursement system. A project to collect data in DDD format for HC is in progress.
Conclusions: AC data collection needs to be extended to other regions of Austria by a comprehensive effort of the health insurers. A complementary data source, providing sales data, will be necessary to ensure a comprehensive data collection.

P1466 Consumption of antibiotics in Belgium: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, date accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).

Methods: In Belgium (10.3 million inhabitants in 2001), reimbursement data (90.5% social coverage) are by law available from community and hospital pharmacies, who transmit to the health insurers and the National Institute of Health Insurance. Medicinal product packages in ambulatory care and distribution units in hospital care all have a unique identifier, linked to the ATC–DDD system. HC data for 2001 are still lacking due to the slow collection and validation process.
Results: AC use increased from 25.4 DID in 1997 to 26.6 DID in 1999 and then declined to 24.3 DID in 2001. Seasonal fluctuationas in AC were high. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides and quinolones (Q) was 9.1, 3.4, 2.9, 3.4, and 3.2 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 2, 37, and 58% of total PEN use, respectively. Belgian AC consumption is characterized by a high use of Q and of amoxillin–clavulanic acid combination. HC use was 2.5 DID in 2000. The proportion of use in 2000 of PEN, CEP, carbapenems, glycopeptides and Q was 50, 19, 1, 1, and 10% of total HC use, respectively.
Strengths and weaknesses: As all antibiotics are reimbursed, this collection system is valid for recording utilization and for expenditures. Delay in routine data provision is 15–24 months.
Conclusions: Although consumption remains high in Belgium, use declined after 1999. Extensive use is made of amoxicillin–clavulanic acid combination and newer antibiotics.

P1467 Consumption of antibiotics in Bulgaria: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Bulgaria (7.9 million inhabitants in 2001), sales data (aggregated for AC and HC) were provided by the Bulgarian Drug Agency for 1999 and 2000. In addition, 5 years consumption data of one hospital (the main multipurpose hospital of Sofia) were available.
Results: Total use was 20.2 DID in 2000. Use of tetracyclines and chloramphenicol was still quite substantial. Use of penicillins (PEN) was predominantly narrow spectrum, ampicillin or amoxicillin. In hospitals, use of third and fourth generation cephalosporins was early and substantial.
Strengths and weaknesses: The comprehensiveness of wholesaler data cannot be guaranteed, nor split for AC and HC. New data collection systems are currently being built, based on reimbursement data for AC and on hospital pharmacists for HC, all using common ATC–DDD methodology.
Conclusions: In Bulgaria, valid estimation of population exposure to antibiotics was possible but without the ability to separate for AC and HC use.

P1468 Consumption of antibiotics in Croatia: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Croatia (4.4 million inhabitants in 2001), sales data were collected by a marketing research company and provided in collaboration by the National Institute of Public Health and by the National Institute of Statistics, with almost 100% coverage for AC and HC, only for 2000 and 2001.
Results: AC use was 18.4 DID in 2000, and 17.6 DID in 2001. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides and quinolones (Q) was 8.6, 3.0, 1.4, 1.8, and 1.3 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 13, 45, and 41% of total PEN use, respectively. HC was 1.9 DID in 2001, and the proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides, and Q was 45, 25, 1, 1, and 9% of total HC use, respectively; the CEP generations represented (from first to fourth) 29, 52, 18, and 1% of total CEP use.
Strengths and weaknesses: AC data are comprehensive, timely and expressed in DID. Historical overview is limited. Data collection in AC and in all individual hospitals is now organized in strong collaboration with the Croatian Committee for Antibiotic Resistance Surveillance.
Conclusions: In Croatia, prospects for appropriate antibiotic use and comprehensive data collection are good.

P1469 Consumption of antibiotics in Czech Republic: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Czech Republic (10.3 million inhabitants in 2001), sales data (aggregated for AC and HC) were provided by the State Institute for Drug Control. In addition, Institute for Health Information and Statistics (Ministry of Health) provided reimbursement data collected by some of the health insurers, covering nearly 100% of insured population, but without guarantee of comprehensiveness. In HC, only one hospital up to now provided data.
Results: Total use of antibiotics remained constant and varied between 18.6 DID in 1997 and 19.7 DID in 2001. Tetracyclines were largely prescribed (3.5 DID in 2001), while quinolones showed most rapid increase during the period of observation.
Strengths and weaknesses: Excellent historical data from the former central wholesaler are available, providing a time series of antibiotic consumption from 1952 till 1990. Individual hospitals participate actively in drug utilization studies, often focused on antibiotics. For AC data collection, collaboration between health insurers needs to be enforced. Support for national coordination between hospital pharmacists could easily spark valid data collection in HC.
Conclusions: In Czech Republic, no valid detailed estimation of population exposure to antibiotics is currently available in the public domain. Unless cooperation between health insurers and hospital pharmacists is supported, probably even made mandatory, this situation will persist.

P1470 Consumption of antibiotics in Denmark: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).

Methods: In Denmark (5.3 million inhabitants in 2001), sales data were collected from the community pharmacies (AC) and hospital pharmacists (HC), and provided by the Danish Medicines Agency.
Results: AC remained constant and varied between 12.2 DID in 1997 and 12.8 DID in 2001. Seasonal fluctuations were small. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides and quinolones (Q) was 8.0, 0.03, 1.0, 2.1, and 0.2 DID, respectively. Narrow spectrum PEN, ampicillin/amoxicillin and combinations with clavulanic acid represented 61, 15, and <1% of total PEN use, respectively.
HC use showed a constant but minor increase, from 1.3 DID in 1997 to 1.4 DID in 2001. In 2001, PEN, CEP, carbapenems, glycopeptides and Q represented 58, 12, 1, 1, and 6% of total HC use, respectively. The different generations of CEP represented (from first to fourth generation) 2, 87, 11, and 0% of total CEP use, respectively.
Strengths and weaknesses: AC data cover the whole country, and the data are linked to the GP and the patient. HC data cover the whole country and are available by ward, but certain antibiotics are not registered (<4%). The quality of the data collection system is high.
Conclusions: In Denmark, AC and HC use remains low. PEN (narrow spectrum in AC) are the most widely prescribed antibiotics.

P1471 Consumption of antibiotics in England: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In England (49.1 million inhabitants in 2001), reimbursement data were provided by the Department of Health, with >95% coverage for AC. No data were available for HC.
Results: AC use varied between 16.8 DID in 1997 and 14.6 DID in 2001, with little seasonal fluctuation. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides and quinolones was 6.6, 0.8, 3.1, 2.2, and 0.4 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin and combination with clavulanic acid was 10, 65 and 12% of total PEN use, respectively.
Strengths and weaknesses: AC data for England are comprehensive, timely and, for the first time, expressed in DID. Cooperation between Wales, Scotland, England, and Northern Ireland will be necessary to provide comprehensive UK data. Linking to the ATC/DDD methodology will be facilitated by the common use of the British National Formulary.
Conclusions: AC consumption in England has been declining steadily over the past four years. Currently, there are no national data about hospital consumption in the UK available and governmental support will be required to improve the situation.

P1472 Consumption of antibiotics in Finland: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Finland (5.2 million inhabitants in 2001), sales data were provided by the National Agency for Medicines.
Results: AC remained constant and varied between 19.4 DID in 1997 and 19.8 DID in 2001; seasonal fluctuations were low. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines (TET), macrolides and quinolones (Q) was 6.1, 2.3, 4.6, 2.2, and 0.8 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 35, 45, and 12% of total PEN use, respectively. HC use showed a constant but minor increase, from 3.5 DID in 1997 to 3.9 DID in 2001. The proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was 14, 20, 1, 1, and 16% of total HC use, respectively; the CEP generations represented (from first to fourth) 36, 50, 13, and 1% of total CEP use.
Strengths and weaknesses: Data cover the whole country, but the split between AC and HC consumption remains to be elucidated.
Conclusion: In Finland, AC use was moderate and TET was largely prescribed. HC use of antibiotics is high in this Scandinavian country, but this may be due to the inclusion of nursing homes and health center wards consumption in HC data.

P1473 Consumption of antibiotics in France: first results of the ESAC retrospective data collection

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Paris, F; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In France (59.0 million inhabitants in 2001), sales data were provided by AFSSAPS (French Health Products Safety Agency) and collected on the basis of mandatory annual reporting of the pharmaceutical companies.
Results: AC use was 33.0 DID in 1997, peaked to 34.3 DID in 1999 and subsequently decreased to 32.9 DID in 2001. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides (M) and quinolones (Q) was, respectively, 16.2, 4.2, 3.1, 6.0, and 2.3 DID; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 1, 59, and 34% of total PEN use, respectively. HC remained constant and was 3.6 DID in 2001. The proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was 49, 7, 0.4, 1, and 10% of total HC use, respectively; the CEP generations represented (from first to fourth) 16, 31, 49, and 4% of total CEP use.
Strengths of the data: Exhaustiveness of antibiotic sales and availability since 1988.
Weaknesses of the data: Only national and annual data without more precise information at a regional or at a monthly or weekly level.
Conclusion: In France, AC and HC use is extremely high. In AC, a very high broad-spectrum PEN, CEP and M consumption was noted. Consumption of antibiotics has stabilized during recent years, but a trend towards decline needs to be confirmed by continuous surveillance of antibiotic consumption.

P1474 Consumption of antibiotics in Germany: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Data were to be collected retrospectively from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).

Methods: In Germany (82 million inhabitants in 2001), AC data were provided by WIdO using a 0.4% sample data base of all compulsory health insurance covered (GKV, 71 million) prescriptions for the years before 2000, and a total GKV prescriptions database for the year 2001. HC data were estimated from the SARI project covering 35 intensive care units (ICUs) located in 17 different regions, and from the MABUSE program covering the medical and surgical services of 8 university hospitals.
Results: AC use declined slightly from 14.5 DID in 1997 to 13.8 DID in 2001. Regional differences ranged between 9.6 and 17.3 DID. In 2001 the use of narrow-spectrum penicillins plus aminopenicillins was 4.1 DID, and the use of tetracyclines was 3.6 DID. The uses of other drug classes (in DID) were: macrolides 2.1; aminopenicillin/betalactamase inhibitor combinations plus oxacillins plus oral cephalosporins, 1.3; and quinolones, 1.1. Between 1997 and 2001, quinolone use was increasing, while tetracycline use decreased. Mean use in ICUs was 133 DDD/100 patient days (range, 71–250). Use ranged between 40 and 80 in the medical service areas outside ICU and between 120 and 150 DDD/100/patient days in the hematology-oncology services. Rough estimates from these data with extrapolation to the 2252 hospitals (169 million patient days) indicate hospital antibiotic use per population to be in an order of magnitude of 1–2 DID.
Strengths and weaknesses: Representative HC data so far are not available while AC data cover ~86% of the total population (excluding private insurance patients). Quarterly AC data have not become available until recently.
Conclusions: In Germany, there has been no major change in overall medical use of antibiotics during the past few years. HC use represents ~10% of the total medical antibiotic use. Penicillin/aminopenicillins and tetracyclines are the most frequently prescribed drug classes.

P1475 Consumption of antibiotics in Greece: first results of the ESAC retrospective data collection

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Athens, GR; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Greece (10.9 million inhabitants in 2001), sales data were provided by the National Organization for Medicines and collected on the basis of mandatory reporting of the pharmaceutical companies.
Results: AC use increased constantly from 25.1 DID in 1997 to 29.4 DID in 2001, which was due to increased use of macrolides (M) and quinolones (Q); high seasonal fluctuations were noted. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, M and Q was 10.1, 6.6, 2.7, 6.9, and 2.1 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid being 5, 56, and 39% of total PEN use, respectively. HC use varied between 2.1 DID in 1997 and 2.2 DID in 2001. The proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was 28, 26, 2, 2, and 9% of total HC use, respectively; the CEP generations represented (from first to fourth) 2, 70, 23, and 5% of total CEP use.
Strengths and weaknesses: AC data covered the entire market, including OTC sales, but also nursing homes, private health care facilities as well as parallel exports; HC data covered all, but not private, hospitals.
Conclusions: In Greece, a constant increase of AC was noted; Q use was high both in AC and HC. However, for valid interpretation and European comparison, parallel export data should be identified.

P1476 Consumption of antibiotics in Hungary: first results of the ESAC retrospective data collection

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Pecs, HUN; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Hungary (9.9 million inhabitants in 2001), complete reimbursement data for the period 1998–2001 were provided by the National Health Insurance (OEP) for AC. For HC, complete sales data (only for 2001) were delivered by the same data provider.
Results: AC use remained rather stable from 18.6 DID in 1998 to 19.1 DID in 2001, with an exceptional high use of 23.9 DID in 1999, possibly related to a serious influenza outbreak during that year. Seasonal fluctuations were high. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides, and quinolones (Q) was 8.7, 2.5, 2.3, 3.3, and 1.2 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 13, 36, and 49% of total PEN use, respectively. HC use was 1.3 DID in 2001. The proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was, 49, 13, 1, <1 and 7‰ of total HC use, respectively; the CEP generations represented (from first to fourth): 4, 87, 8, and 1% of total CEP use.
Strengths and weaknesses: AC data were relatively accurate since they covered 100% of the population and were based on doctor's prescription. The hospital consumption, only available since 2001, will be improved by a broader usage of electronic data collection in the future.
Conclusions: In Hungary, a relative constant use of antibiotics was observed in AC. Continuation of data collection in HC will need logistic support.

P1477 Consumption of antibiotics in Iceland: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Iceland (0.3 million inhabitants in 2001), total sales data from pharmaceutical companies were provided by the Ministry of Health. No differentiation between AC and HC use could be made.
Results: Total use slowly decreased from 21.5 DID in 1998 to 19.1 DID in 2001. Seasonal fluctuations are small except for the winter of 1998 with a peak consumption of 27.3 DID. Penicillins (PEN) accounted for half of the total antibiotic use. In 2001, total use of PEN, cephalosporins (CEP), tetracyclines, macrolides, and quinolones (Q) was 10.3, 0.5, 4.7, 1.6, and 0.7 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 29, 35, and 20% of total PEN use, respectively.
Strengths and weaknesses: Complete coverage of all antibiotics sold in Iceland was obtained. However, it is impossible to differentiate between AC and HC use.
Conclusions: Use of antibiotics in Iceland is rather high, slowly decreasing and mainly consisting of penicillins. The data collection system is hampered by the aggregation of AC and HC data.

P1478 Consumption of antibiotics in Italy: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and

hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Italy (57.5 million inhabitants in 2001), sales data per year for the period 1999–2001, covering 90% of population, were provided by the Ministry of Health (Direzione Generale della Valutazione dei Medicinali e della Farmacovigilanza). Prescribed, non reimbursed as well as OTC antibiotics were included. For HC, data were collected from one hospital for the period 1997–2000 and from six hospitals for 2001.
Results: AC use varied between 25.0 DID (1999) and 27.3 DID (2001). AC use is characterised by a high consumption of penicillins (PEN). In 2001, use of PEN, cephalosporins (CEP), tetracycline, macrolides (M) and quinolones (Q) was, 11.6, 3.5, 0.5, 5.1, and 3.8 DID, respectively; the proportions of narrow spectrum PEN, ampicillin/amoxicillin, and amoxicillin plus clavulanic acid were, <1, 52, and 39%, respectively, of total PEN use. In HC, the proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was 19, 15, 1, 3 and 9% of total HC use, respectively; the CEP generations represented (from first to fourth) 20, 3, 72, and 5% of total CEP use. An extremely high use of colistin (J01XB01) was observed.
Strengths and weaknesses: AC data were comprehensive but a delay in delivery was observed. HC data were not yet representative for the whole of Italy.
Conclusion: In Italy, a high AC consumption was observed with a high use of Q. For HC data collection, the sample of hospitals needs to be enlarged.

P1479 Consumption of antibiotics in Latvia: first results of the ESAC retrospective data collection

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Riga, LV; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Latvia (2.4 million inhabitants in 2001), only 2001 sales data from wholesalers were provide by the State Medicinal Agency, separately for AC and HC. Validation of the use of ATC-methodology, comprehensiveness of the data, and details on the split between AC and HC could not be assessed.
Results: AC use was 13.1 DID in 2001. In that year, the use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides and quinolones (Q) was 10.4, 0.6, 0.1, 0.3, and 0.6 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 1, 84, and 15% of total PEN use, respectively.
HC consumption was 4.5 DID in 2001, and the proportion of PEN, CEP, carbapenems, glycopeptides, and Q was 86, 6, <1, <1, and 1% of total HC use, respectively; the CEP generations represented (from first to fourth) 70, 6, 22, and 2% of total CEP use.
Strengths and weaknesses: Data are provided on a yearly basis only. No major changes in data collection performance are anticipated for the near future.
Conclusions: In Latvia, no validated, longitudinal estimation of population exposure to antibiotics is currently available in the public domain. The proportion of HC use on total exposure appears to be exceptionally high. Narrow spectrum PEN, ampicillin and amoxicilin dominate antibiotic consumption, both in AC and in HC.

P1480 Consumption of antibiotics in Lithuania: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Lithuania (3.7 million inhabitants in 2001), AC data (provided by the State Patient Fund) are not comprehensive, because of the complex nature of the reimbursement status of antibiotics (only a limited number of antibiotics are reimbursed, only for special categories of patients and certain diseases). HC data stem from a sample of 5 hospitals, which cover up to 15% of total patient days.
Results: The proportion of HC use in 2001 of penicillins, cephalosporins (CEP), carbapenems, glycopeptides and quinolones was 55, 12, 2, 1, and 2% of total HC use, respectively; the CEP generations represented (from first to fourth) 18, 62, 15, and 5% of total CEP use.
Strengths and weaknesses: Unless legislative changes make the provision of sales data from wholesalers or community pharmacists possible, no valid AC data collection will be possible. For HC, the current sample of hospitals needs to be enlarged.
Conclusions: In Lithuania, no valid estimation of population exposure to antibiotics is currently available from existing databases in the public domain.

P1481 Consumption of antibiotics in Luxemburg: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Luxemburg (0.4 million inhabitants in 2001), reimbursement data for AC were provided by the National Health Insurance Company; HC data were collected by the hospital pharmacists.
Results: AC use varied between 25.8 DID in 1997 and 26.4 DID in 2001. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines macrolides and quinolones (Q) was 9.8, 4.9, 2.6, 4.7, and 2.5 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 2, 43, and 52% of total PEN use, respectively. HC use remained constant and was 2.0 DID in 1997 and 2.1 DID in 2001. The proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was 42, 30, 2, 1, and 10% of total HC use, respectively; the CEP generations represented (from first to fourth) 18, 62, 15, and 5% of total CEP use.
Strengths and weaknesses: AC data are prescription data which covered 96.1% of the total population, i.e. those insured at the national Health Insurance. HC data covered 89.5% of the hospital occupancy.
Conclusion: In Luxemburg AC use is high, particularly of ampicillin/amoxicillin and the combination with clavulanic acid.

P1482 Consumption of antibiotics in Malta: first results of the ESAC retrospective data collection

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G'Mangia, MT; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).

Methods: In Malta (0.4 million inhabitants in 2001), no AC data are available. For HC, comprehensive data are collected by the Government Pharmaceutical Services, covering all public hospitals and 97% of the private hospitals.
Results: HC use was 2.5 DID in 1997, peaked with 4.1 DID in 1999 and was 3.0 DID in 2001. The proportion of HC use in 2001 of penicillins, cephalosporins (CEP), carbapenems, glycopeptides, and quinolones was 55, 12, <1, <1, and 6% of total HC use, respectively; the CEP generations represented (from first to fourth) 26, 51, 23, and 0% of total CEP use.
Strengths and weaknesses: No valid AC data collection will be available, unless legislative changes are made.
Conclusions: In Malta, no valid estimation of population exposure to antibiotics is currently available in the public domain, despite comprehensive data collection in hospitals.

P1483 Consumption of antibiotics in the Netherlands: first results of the ESAC retrospective data collection

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Sittard, Rotterdam, NL; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, the accessibilities, strengths and weaknesses of national systems were assessed and quarterly data were collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries. The ATC/DDD classification (WHO, version 2002) was used, and data were expressed in DDD/1000 inhabitants per day (DID).
Methods: In the Netherlands (16.0 million inhabitants in 2001), AC sales data were collected and analyzed by the Foundation of Pharmaceutical Statistics (SFK) and provided by SWAB; data from a 88% sample of community pharmacies were weighted and extrapolated. For HC, data were requested by SWAB from all Dutch hospital pharmacists; 60 hospitals responded (62% bed days) and data were extrapolated.
Results: AC use remained surprisingly constant at the low level of 10.0 DID (the lowest in Europe) from 1997 to 2001, with little seasonal fluctuations. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides and quinolones (Q) was 3.9, 0.7, 2.4, 1.3, and 0.9 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 10, 46, and 31% of total PEN use, respectively. In HC, antibiotic use remained constant at 0.8 DID. The proportion of use in 2000 of PEN, CEP, carbapenems, glycopeptides, and Q was 55, 11, 1, 1, and 9% of total HC use, respectively; the CEP generations represented (from first to fourth) 22, 48, 28, and 2% of total CEP use.
Strengths and weaknesses: HC data collection was cumbersome. Validation with other available data sources on AC within health insurers is still to be performed.
Conclusions: In the Netherlands, use of antibiotics in AC and HC is low, constant, and apparently appropriate. Integration of data collection of multiple sources through national collaboration is on its way.

P1484 Consumption of antibiotics in Norway: first results of the ESAC retrospective data collection

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Oslo, N; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2001), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Norway (4.5 million inhabitants in 2001), total sales data were provided by the National Institute of Public Health. For 1998 and 2001, separate HC use data were available and differentiation between AC and HC use could be made by subtracting HC use from total use.
Results: Total use remained stable around 17 DID. In 2001, AC use was 15.7 DID and use of penicillins (PEN), cephalosporins(CEP), tetracyclines, macrolides, and quinolones (Q) was, 6.7, 0.3, 3.1, 1.8, and 0.4 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 66, 13, and <1% of total PEN use, respectively. HC use was 1.1 DID in 2001. The proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was: 47, 23, 1, <1, and 4% of total HC use, respectively; the CEP generations represented (from first to fourth): 26, 49, 25, and 0% of total CEP use.
Strengths and weaknesses: Total Norwegian data are complete and comprehensive. A prescription database is planned for 2003, with delivery from all pharmacies to the National Institute of Public Health.
Conclusions: Norway had a stable and low use of antibiotics with a high proportion of narrow spectrum penicillins used. The new data collection system will enable the differentiation between AC and HC use.

P1485 Consumption of antibiotics in Poland: first results of the ESAC retrospective data collection

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Warsaw, PL; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Poland (38.6 million inhabitants in 2001), sales data were provided by the National Institute of Public Health, for AC as well as for HC. Data for ESAC were derived from reports of 200 out of 400 wholesalers (covering about 60% of the market) and were extrapolated for complete population coverage.
Results: AC use sharply increased from 16.6 DID in 1997 to 24.8 DID in 2001, with an increasing high use of penicillins (PEN), a high use of tetracyclines and a low use of macrolides. Seasonal fluctuation is high. In 2001, use of PEN, cephalosporins (CEP), tetracyclines, macrolides, and quinolones (Q) was 11.1, 2.3, 4.2, 2.6, and 1.0 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 10, 70, and 19% of total PEN use, respectively. HC varied between 2.7 DID in 1997 and 2.4 DID in 2001. The proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was 41, 19, <1, <1, and 6% of total HC use, respectively; the CEP generations represented (from first to fourth): 8, 60, 32, and 0.3% of total CEP use.
Strengths and weaknesses: Although data for all years could be provided, the completeness of the data from the first years of the observation period could be questioned. Data are based on reports of half of the wholesalers covering 60% of the sales. Main characteristics of these wholesalers need to be made available for precise extrapolation.
Conclusions: In Poland, total AC use of antibiotics increased rapidly while HC use remained stable. Particularly a growing use of penicillins in AC was observed. Since data was derived from one-half of wholesalers, careful extrapolation is needed.

P1486 Consumption of antibiotics in Portugal: first results of the ESAC retrospective data collection

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Lisbon, P; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).

Methods: In Portugal (10.0 million inhabitants in 2001), reimbursement data for AC, covering 75% of the population, were provided by the Ministry of Health. For HC, only reimbursement data of 1998 could be delivered.
Results: AC use was at a high level of 24.6 DID in 2001, with an exceptional high use of quinolones (Q). In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides, and quinolones (Q) was 11.7, 3.1, 1.4, 3.7, and 3.7 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 1, 35, and 59% of total PEN use, respectively.
Strengths and weaknesses: AC data were relatively accurate since all antibiotics are included in the reimbursement system. However, data were delivered in a format, which was difficult to analyze. HC data were only available for 1998 and were collected within the framework of a national survey. End 2002, a nationwide on-line collection network will be finalized, enabling data collection on a monthly base from all hospitals included in the national health service.
Conclusions: In Portugal, AC use of antibiotics is rather high. Particularly for HC, a new system of data collection needs to be established in order to obtain comprehensive HC use data.

P1487 Consumption of antibiotics in Slovakia: first results of the ESAC retrospective data collection

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Bratislava, SK; Antwerp, B

Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Slovakia (5.4 million inhabitants in 2001), wholesalers data were provided by the Slovak Institute for Drug Control, split for AC and HC, on a monthly basis, since 1999.
Results: AC use was 25.7 DID in 1999 and 24.1 DID in 2001, with considerable seasonal fluctuations. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides and quinolones was 14.1, 2.0, 2.1, 3.3, and 1.5 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 27, 44, and 29% of total PEN use, respectively.
HC use was 1.4 DID in 2001. In that year, the proportion of use of PEN, CEP, carbapenems, glycopeptides, and Q was 40, 20, <1, <1, and 15% of total HC use, respectively; the CEP generations represented (from first to fourth) 18, 51, 31, and <1% of total CEP use.
Strengths and weaknesses: The wholesaler data are comprehensive, timely and monthly, but cannot be used for regional and clinically oriented analysis.
Conclusions: In Slovakia, antibiotic use is high in AC and relatively low in HC.

P1488 Consumption of antibiotics in Slovenia: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Slovenia (2.0 million inhabitants in 2001), reimbursement data were provided by the Institute of Public Health with 100% coverage for AC. In HC, hospital pharmacists provided the data. The coverage of bed days was between 1998 and 2001, 85, 89, 98, and 100%, respectively.
Results: AC use was 17.5 DID in 1997, with a peak at 19.8 DID in 1999, again falling to 17.4 DID in 2001, with moderate seasonal variation. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides and quinolones was 10.2, 0.5, 0.8, 3.3, and 1.3 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was 26, 30, and 43% of total PEN use, respectively. HC use varied between 1.9 DID in 1998 and 1.8 DID in 2001. In HC, the proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides, and Q was 43, 19, 1, 1, and 14% of total HC use, respectively; the CEP generations represented (from first to fourth) 26, 31, 43, and 0% of total CEP use.
Strengths and weaknesses: AC data collection is comprehensive and timely. HC data had complete coverage in 2001, but was incomplete for the previous years.
Conclusions: In Slovenia, antibiotic use in AC and HC was moderate and slightly declining in the last two years.

P1489 Consumption of antibiotics in Spain: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Spain (39.9 million inhabitants in 2001), reimbursement data for AC were provided by Spanish Drug Agency and obtained from the ECOM Database of the Ministry of Health; HC data were provided by the Society of Hospital Pharmacists, and included 15% of hospitals (predominantly big hospitals).
Results: AC decreased constantly from 21.4 DID in 1997 to 18.8 DID 2001. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines, macrolides (M) and quinolones (Q) was 9.9, 2.2, 0.7, 3.1, and 2.5 DID, respectively; the proportion of narrow spectrum PEN, ampicillin/amoxicillin, and combination with clavulanic acid was, 1, 48, and 48% of total PEN use, respectively. In HC, the proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was 47, 15, 2, 2, and 14% of total HC use, respectively; the CEP generations represented (from first to fourth) 21, 36, 39, and 4% of total CEP use.
Strengths and weaknesses: Although the ECOM AC database covers all prescriptions of the Spanish Social Security System, it underestimates antibiotic use in Spain (OTC, prescriptions covered by private insurance companies, and veterinary prescriptions not included).
Conclusion: Although AC use is high in Spain, particularly of the Q, a constant decrease was noted since 1997. Sampling in HC needs to be enlarged and stratified to produce a valid estimation of population exposure.

P1490 Consumption of antibiotics in Sweden: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Sweden (8.8 million inhabitants in 2001), sales/prescription data were provided by the National Corporation of Swedish Pharmacies.
Results: AC use remained constant and varied between 14.6 DID in 1997 and 15.8 in 2001; seasonal fluctuation was low. In 2001, use of penicillins (PEN), cephalosporins (CEP), tetracyclines (TET), macrolides (M) and quinolones (Q) was 7.5, 0.5, 3.3, 1.0, and 1.1 DID, respectively; the proportion of narrow

spectrum PEN, ampicillin/amoxicillin and combination with clavulanic acid was 64, 12, and 3% of total PEN use, respectively. HC use remained constant and varied between 1.26 DID in 1997 and 1.29 DID in 2001. The proportion of use in 2001 of PEN, CEP, carbapenems, glycopeptides and Q was 35, 19, 2, 1, and 14% of total HC use, respectively; the CEP generations represented (from first to fourth) 13, 73, 14, and 0.3% of total CEP use.
Strengths and weaknesses: Data cover the complete consumption in Sweden. The quality of the data collection system is high.
Conclusion: In Sweden, both AC and HC use is low; in AC, narrow spectrum PEN are used extensively, whereas in HC, second generation CEP are frequently prescribed.

P1491 Consumption of antibiotics in Turkey: first results of the ESAC retrospective data collection

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Objectives: European Surveillance of Antibiotic Consumption, granted by DG SANCO of the EC (ESAC) is an international network of national surveillance systems, aiming to collect comparable antibiotic consumption data in Europe. During the first phase, data accessibility and validity, as well as strengths and weaknesses of national systems were assessed. Quarterly data were to be collected retrospectively (1997–2001) from ambulatory (AC) and hospital care (HC) in 31 countries, using ATC/DDD classification (WHO, version 2002), and expressing results in DDD/1000 inhabitants per day (DID).
Methods: In Turkey (67.6 million inhabitants in 2001), only incomplete sales data expressed in units were available from Information Medical Statistics (IMS) Health for AC.
Results: Available data suggest a high antibiotic consumption, slowly declining over the last five years, with considerable use of broad-spectrum antibiotics and pronounced seasonal variation in prescribing.
Strengths and weaknesses: HC data are absent. Health insurers are building real time online connections to a great number of community and hospital pharmacies in this vast country, from which timely and comprehensive data collection could be possible, provided privacy issues are adequately dealt with.
Conclusions: No valid estimation of population exposure to antibiotics is available in the public domain for Turkey. Application of ATC/DDD methodology needs to be considered for future web-based data collection.

Anti-fungals in vitro susceptibility

P1492 A new agardilution assay for testing voriconazole against isolates of *Aspergillus* spp.: a comparison with the NCCLS microdilution reference method

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Objectives: The available National Committee for Clinical Laboratory Standards (NCCLS) microdilution method for the antifungal susceptibility testing of filamentous Fungi may not be the most efficient and convenient procedure for use in the clinical laboratory. We developed an easy to read susceptibility test assay, using solid RPMI 1640 Agar containing different drug concentration. The purpose of this study was to evaluate this new method for susceptibility testing of *Aspergillus* isolates to the new triazole voriconazole.
Methods: We compared MIC values obtained simultaneously by agardilution and NCCLS broth microdilution methods after 24- and 48-h of incubation for reference the novel triazole voriconazole. The 25 clinical isolates evaluated included 15 *A. fumigatus*, and 10 *A. niger*. The MICs of voriconzole in the agardilution method corresponded to the first well with no growth.
Results: Three comparisons of MIC pairs by the two methods were evaluated to obtain percentages of agreement (±1 dilution range): 24- and 48-h MIC by agardilution vs. corresponding 24- and 48 h NCCLS MICs and 24-h agardilution vs. 48-h reference MICs. The agreement between the methods for voriconazole MICs was 92% when both 24- and 48-h agardilution MICs were compared with 24- and 48-h NCCLS MICs. The agreement was slightly higher (96%) when 24-h agardilution values were compared with 48-h NCCLS MICs.
Conclusion: These data suggest the potential value of the agardilution assay for use in the clinical laboratory to test the susceptibilities of common *Aspergillus* isolates to voriconazole.

P1493 In vitro activity of flucytosine, caspofungin, voriconazole, and amphotericin B in 2-drug combinations against *Aspergillus* spp.

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Objectives: To assess whether two-drug combination will increase the in vitro activity of antifungals active against *Aspergillus* spp. and the potential effectiveness of 5FC in this setting.
Methods: A two-dimensional checkerboard based on the NCCLS M-38P microdilution broth technique was used. Two-drug combinations of flucytosine (5FC) with caspofungin (CAS) or voriconazole (VRZ) were evaluated against 14 recent clinical isolates of *Aspergillus* spp. (12 *A. fumigatus* and 2 *A. terreus*). Combination of CAS with amphotericin B (AMB) or VRZ were tested against 35 isolates (30 *A. fumigatus* and 5 *A. terreus*). After 48 h of incubation microplates were read visually and MICs were defined as the lowest concentration that gave 50% (CAS, 5FC, VRZ) or 100% (AMB) of inhibition. FIC indices (FICI) were calculated and interactions were classified as synergistic (FICI $\leq$0.5), additive (FICI $>$ 0.5 and $\leq$1), indifferent (FICI $>$ 1 and $\leq$4), and antagonistic (FICI $>$ 4). All experiments were run in duplicate.
Results: (Table):

	% of *A. fumigatus* strains			
Interaction	5FC + CAS (n = 12)	5FC + VRZ (n = 12)	CAS + AMB (n = 30)	CAS + VRZ (n = 30)
Synergistic	92	0	28	9
Additive	0	0	66	68
Indifferent	8	42	6	23
Antagonistic	0	58	0	0

For the synergistic interactions the median concentrations of CAS and 5FC in combination were 0.25 μg/mL (range: 0.25–4 μg/mL) and 4 μg/mL (range: 2–16 μg/mL), respectively. In *A. terreus* this combination was synergistic for one strain.
Conclusions: Combination of CAS with either AMB or VRZ was mostly additive, while a marked synergy was observed for the combination of CAS with 5FC. On the other hand, combination of VRZ with 5FC was indifferent to antagonistic. These results warrant further investigation in animal models of aspergillosis.

P1494 In vitro interaction of caspofungin with amphotericin B against clinical isolates of *Trichosporon asahii*

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Objective: Invasive trichosporonosis is often difficult-to-treat and associated with high mortality. Currently available antifungal agents frequently have limited or no activity against the infecting fungus and clinical failure following treatment with amphotericin B or the azoles is not uncommon. The novel echinocandin, caspofungin, is also known to lack meaningful activity against *Trichosporon*. We investigated whether the combination of caspofungin and amphotericin B in vitro would yield more enhanced activity than each drug alone against clinical isolates of *Trichosporon asahii*.
Methods: A total of 69 *T. asahii* strains were included in the study. The individual caspofungin and amphotericin B MICs (μg/mL) were determined by using NCCLS M27-A2 microdilution method. In vitro interaction of

caspofungin and amphotericin B was explored by using checkerboard method and determination of fractional inhibitory concentrations (FIC; μg/mL). MIC-0 endpoint was used for both MIC and FIC readings and the results were read at 24 and 48 h. The combination test results were evaluated by calculating the FIC index (FICI) and interpreted as follows: FICI $\leq$0.5, synergistic; 0.5 < FICI $\leq$1, additive; 1 < FICI $\leq$4, indifferent; and FICI > 4,
Results: At 24 h, caspofungin and amphotericin B were synergistic, additive, and indifferent for 3 (4.4%), 17 (24.6%), and 49 (71%) isolates, respectively. At 48 h, the two drugs were synergistic, additive, and indifferent for 9 (13%), 28 (40.6%), and 32 (46.4%) isolates, respectively. Antagonistic interaction was not detected.
Conclusions: (i) Caspofungin and amphotericin B tend to be synergistic, additive or indifferent in vitro against *T. asahii*. Importantly, antagonism is not observed in any of the isolates. (ii) At 48 h, synergistic or additive interaction are observed at a significantly higher rate compared with 24 h (54 vs. 29%, respectively). (iii) These in vitro data appear promising for potential use of caspofungin and amphotericin B combination in a number of infections due to *T. asahii*. Clinical implications and significance of these findings demand to be validated by in vivo investigations.

P1495 Effect of pH on the in vitro activity of amphotericin B and flucytosine against yeasts and moulds

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Background: The efficacy of antifungal agents in treating invasive fungal infections is limited due to several reasons. One reason could be physical conditions at the side of infection including pH. Since MICs are determined at pH 7, altered activity of drugs at other pH values will not be detected.
Objectives: To determine the effect of pH on the in vitro activity of amphotericin B (AMB) and flucytosine (5FC) against yeasts and moulds.
Methods: Susceptibility of four yeast isolates (two *Candida krusei* and two *Cryptococcus neoformans*) and six mould isolates (two *Aspergillus fumigatus*, two *Rhizopus* spp. and two Scedosporium prolificans) to AMB and 5FC was tested in triplicate by the NCCLS broth microdilution method. Susceptibility testing was performed in RPMI 1640, adjusted to pH values ranging from 4.0 to 7.9. Buffering was achieved with citrate (10 mM), citrate-phosphate (10 mM) or morpholinepropanesulfonic acid (MOPS; 0.165 M). The median MIC (MIC50) was calculated for each isolate and pH.
Results: For all yeast isolates, *A. fumigatus* isolates and *Rhizopus* spp. the MIC50 of AMB decreased when the medium pH increased. The MIC50 was between 32 and 64 mg/L (depending on the tested isolate) at pH 4.0, while at pH 7.9 the MIC50 was between 0.016 and 0.5 mg/L. For the *S. prolificans* isolates a MIC50 equal to or higher then 32 mg/L was found, independent on the medium pH. For all yeast isolates and *A. fumigatus* isolates the MIC50 of 5FC increased when the medium pH increased. The MIC50 of 5FC varied between 0.031 and 1 mg/L (depending on the tested isolate) at pH 4.0, while at pH 7.9 the MIC50 varied between 4 and 1024 mg/L. For the *Rhizopus* spp. and the *Scedosporium prolificans* isolates a MIC50 > 1024 mg/L was found.
Conclusions: The in vitro activity of both AMB and 5FC against yeasts and moulds depends on the medium pH. Susceptibility of AMB decreased with lowering pH, while the susceptibility of 5FC increased with lowering pH. Because the pH at the site of infection may be relatively low, these findings may have important consequences for therapy.

P1496 Biofilm production and antifungal susceptibility patterns of *Candida* spp.

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Objectives: Biofilms serve as a nidus for an infectious disease and are often associated with antimicrobial resistance of microorganisms. In this study, biofilm production and antifungal susceptibility of various *Candida* species were examined and compared.
Methods: A total number of 165 *Candida* species (99 *C. albicans*, 22 *C. tropicalis*, 20 *C. glabrata*, 12 *C. parapsilosis*, 10 *C. krusei*, 1 *C. guilliermondii* and 1 *C. kefyr*) isolated from different clinical specimens were included in this study. The biofilm production of the strains was searched by modified tube adherence test and their antifungal susceptibilities against fluconazole and amphotericin B were determined by microdilution method performed according to NCCLS M27-A standards.
Results: Forty-five (27.3%) of the strains were found to be slime producing. The rate of biofilm formation by different species ranged between 16.7 and 60%. No significant difference was found between the biofilm production of *C. albicans* and non albicans species ($X^2 = 2.04$; $P = 0.15$). MIC50 and MIC90 values for fluconazole ranged between 4 and 64 μg/mL and 32–>64 μg/mL for different *Candida* species while these values changed between 0.25 and 1 μg/mL and 0.5–2 μg/mL for amphotericin B, respectively. Forty-eight (29.1%) and 24 (14.5%) of the isolates were found to be dose dependent susceptible and resistant to fluconazole, respectively. Eleven (6.7%) of the strains were detected to be resistant to amphotericin B. When the relation between the biofilm production and the susceptibility categories of the strains were searched, statistical differences were found ($P = 0.00$ and $P = 0.02$) which meant that biofilm production and susceptibility categories were not parallel.
Conclusion: It can be concluded that biofilm production is a potential virulence factor for *Candida* species. However this factor does not seem to correlate with the susceptibility categories of the strains, when the planktonic cells are used for the susceptibility testing. Antifungal susceptibility of the sessile cells from biofilms should also be explored.

P1497 In vitro susceptibility testing of voriconazole and fluconazole: Effects of incubation time and culture medium, and the 'trailing' phenomenon

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Objectives: This study aimed at investigating the dependency of antifungal susceptibility testing on the incubation time and the culture medium. Furthermore the so-called 'trailing' phenomenon (residual growth at concentrations greater than the MIC) was investigated.
Methods: In a collaborative study involving 12 centers, microdilution, *E*-test and agar diffusion tests with voriconazole and fluconazole were performed. Two thousand thirty-three freshly isolated yeasts and 23 control strains were tested according to DIN 58940–84 (microdilution) and DIN 58940–3 (agar diffusion) with Yeast Susceptibility Test medium (YST, Sifin GmbH, Berlin) and in part (agar diffusion) also with Mueller Hinton medium supplemented with 2% glucose and 0.5 mg/L methylene blue (MH + MB). Results were read within 24, 48 and 72 h. Minimal inhibitory concentration (MIC) readings were performed visually; inhibition zone diameter (IZD) determination was done with an automated video system (BIOMIC, Giles Scientific, USA).
Results: Within 24 h, a definite endpoint could be determined at the following percentages for voriconazole and fluconazole: 84 and 82% with microdilution using YST; 79 and 78% with *E*-test using YST; 77 and 77% with agar diffusion using YST; 58 and 57% with agar diffusion using MH + MB. The trailing phenomenon was reported at the following percentages: 22 and 25% with microdilution using YST; 31 and 34% with *E*-test using YST; 13 and 15% with agar diffusion using YST; 39 and 41% with agar diffusion using MH + MB. Trailing was reported about 10% more frequently at 48 h, and significantly less frequently at 72 h (4–12%). Test results with *Cryptococcus* spp., *Rhodotorula* spp. *C. guilliermondii*, *C. parapsilosis*, *C. famata* and *Trichosporon asahii* were usually not interpretable at 24 h; these species require incubation of 48 h.
Conclusion: YST medium according to DIN 58940 is suitable for microdilution and agar diffusion testing (conventional test disks and *E*-test) of voriconazole and fluconazole. The trailing phenomenon is reduced as compared with supplemented Mueller-Hinton medium, and comparable and reproducible SIR assessments within 24 h is possible for most yeast species.

P1498 Evaluation of ketoconazole and fluconazole susceptibility testing with disk-diffusion method on three different culture media

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Objectives: The present study was carried out to compare three different culture media for antifungal susceptibility testing using disk-diffusion method with *E*-test as reference method.
Materials and methods: The tested 45 *Candida* strains were isolates from women with acute or recurrent vulvovaginal candidosis. Strains were collected in the microbiology laboratory of Tartu University Clinics, Estonia. Strains were identified using routine methods as *C. albicans* ($n = 27$), *C. krusei*

($n = 13$) and *C. glabrata* ($n = 5$). The tested antifungal disks were fluconazole (25 μg) and ketoconzole (10 μg; MAST Diagnostics, UK). The synthetic medium RPMI 1640 supplemented with L-glutamin and 2% glucose (Angus Biochemicals, USA), Yeast Nitrogen Base Medium (YNB, Difco, USA) and Mueller-Hinton Agar (Oxoid, UK) supplemented with methylene-blue was used for disk-diffusion. The *E*-test ranges for tested antifungals were 0.016–256 mg/L for fluconazole and 0.016–256 mg/L for ketoconzole (AB Biodisk, Sweden). The synthetic medium RPMI 1640 was used for *E*-test. The results were read after 24 and 48 h accordingly. The inoculum was prepared from colonies incubated on Sabouraud glucose agar for 24 h at 37°C and stock suspension with a density equivalent to 0.5 McFarland was used.

Results: All strains were susceptible for ketoconazole (MIC 0.002–1.5 mg/L), 60% of stains were susceptible, 16% were susceptible dose-dependant and 24% were resistant for fluconazole (MIC 0.02–>256 mg/L). Comparing disk-diffusion results (inhibition zone mm) on different media with *E*-test (MIC values mg/L) we found best correlation in case of Mueller-Hinton agar and incubation time 24 h. For fluconazole the correlation coefficient (r) was -0.85 and for ketoconazole $r = -0.81$ ($P < 0.001$). The results after 48 h incubation did not differ significantly from 24 h ones. For YNB incubated for 24 h correlation with *E*-test was lower (fluconazole $r = -0.57$; $P < 0.001$; ketoconazole $r = -0.42$; $P < 0.01$). All other medium-incubation time combinations did not gave reliable results comparing with *E*-test.

Conclusions: Disk-diffusion test on Mueller-Hinton Agar supplemented with methylene-blue could be recommended for susceptibility testing of yeasts as cost-effective alternative for MIC testing. Shorter incubation time (24 h instead of 48 h) using this medium gives opportunity for earlier treatment.

P1499 In vitro activities of voriconazole, amphotericin B and fluconazole against *Candida* strains isolated from neutropenic patients with hematologic malignancies

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The incidence of invasive fungal infections has increased in the last 20 years also have witnessed an increased resistance to established antifungal agents. Among the new azole, voriconazole (UK-109, 496; Vfend [Pfizer Pharmaceuticals, New York]) is a new triazole that has not proposed standard procedures for antifungal susceptibility testing methods. In this study, we determined minimal inhibitor concentrations of voriconazol, amphotericin B and fluconazole by following the NCCLS M27-A broth microdylution method. A total of 111 clinical *Candida* isolates, including isolates of *Candida albicans* (52), *Candida glabrata* (20), *Candida tropicalis* (13), *Candida parapsilosis* (3), *Candida kefiy* (18), *Candida famata* (1), *Candida krusei* (1) were included in our study. The voriconazole MIC at which 50% of strains were inhibited (MIC 50) was 0.5 μg/mL for *C. albicans*, it was 0.007 μg/mL for *C. glabrata*, 0.5 μg/mL for *C. tropicalis*, 0.007 μg/mL for *C. kefyr*, 1 μg/mL for *C. parapsilosis*. All the tested isolates were inhibited by voriconazole at concentrations ranging from 0.007 to 2 μg/mL. Voriconazole had consistent and significant activity against isolates of both *C. krusei* and *C. glabrata*, as opposed to fluconazole. MIC 50 of fluconazole was 1 μg/mL for *C. albicans* 32 μg/mL for *C. glabrata*, 32 μg/mL for *C. tropicalis*, 0.25 μg/mL for *C. kefyr*, 1 μg/mL for *C. parapsilosis*. MIC 50 of amphotericin B was 0.0313 μg/mL for *C. albicans*. MICs of fluconazole for *C. glabrata* were very high. (MIC 50 = 32 μg/mL *C. tropicalis* isolates surprisingly showed high fluconazole MIC values ranging from 0.25 to 128 μg/mL. The voriconazole MICs for *Candida* isolates covered a narrow range, with no prominent difference among species. All *Candida* species appeared potentially susceptible to voriconazole, including isolates of *C. krusei*, and *C. glabrata*.

P1500 In vitro susceptibility of *Candida* species isolated from clinical specimens against some antifungal agents

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Objective: Antifungal drug resistance is becoming a major problem in certain populations, especially in immunosuppressed patients. The aim of this study was determine to resistance of *Candida* species isolated from blood cultures of cancer patients and oropharyngeal swabs of diabetic patients to ketoconazole (KET), fluconazole (FLU), itraconazole (ITRA), amphotericin B (Amp B), terbinafine (TRB), flucytosine (FCU). The isolates were obtained from the hospitalized patients in Ankara University, Faculty of Medicine, Department of Hematology, Medical Oncology and Endocrinology.

Material and methods: The most commonly identified species of *Candida* were *C. albicans* (56) (22 of diabetic patients isolates), followed by *C. parapsilosis* (7), *C. tropicalis* (3) (of diabetic patients isolates), *C. guillermondii* (2) and *C. pelliculosa* (1), respectively. An adapted NCCLS M 27-A method was used to evaluate the activity of KET, FLU, ITRA, Amp B, TRB and FCU. The MICs of the strains were done using RPMI 1640 medium by microdilution method.

Results: There were no isolates of *C. parapsilosis*, *C. guillermondi*, *C. pelliculosa* resistant to KET and TRB. There were slight differences in the susceptibility patterns of 13 nonalbicans *Candida* spp. isolates. The isolates *C. pelliculosa* showed intermediate resistance to FLU (MIC to FLU 31.25 μg/mL). Resistance to KET appeared in 1 of *C. tropicalis* from diabetic isolates and followed by 2 FLU, 1 ITRA, 1 Amp B and 3 FCU. Resistance to KET appeared in eight strains of *C. albicans* from blood isolates followed by 11 FLU, 24 ITRA, 8 Amp B. Resistance to the diabetic isolates appeared in 1 KET, 14 FLU, 15 ITRA, 10 Amp B and 20 FCU. Resistance to ITRA appeared in 40 strains of *C. albicans* (MIC to ITRA > 1 μg/mL), 2 strains of *C. parapsilosis*, 2 strains of *C. guillermondi* 1 strain of *C. tropicalis* and 1 strain of *C. pelliculosa*. Resistance to FCU appeared in 49 strains of *C. albicans* (MIC to FLU > 32 μg/mL). So among of the 69 isolates, 22% were resistant to KET, 41% to FLU, 58% to ITRA, 39% to Amp B and 62% to FCU

Conclusion: The main conclusion of this study is that typing and antifungal susceptibility pattern will contribute to the prevention of invasive fungal infections in special hosts such as immunosuppressive patients and diabetic patients.

P1501 Antifungal susceptibility of bloodstream isolates of *Candida* spp. from Kuwait

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Objectives: Nosocomial candidiasis is a major problem in critically ill, high-risk patients with an estimated attributable mortality of 38%. This study presents antifungal susceptibility data on 308 bloodstream isolates of *Candida* (*C. albicans* – 111, *C. parapsilosis* – 128, *C. tropicalis* – 44, *C. krusei* – 13, *C. glabrata* – 12) against four antifungal agents.

Methods: The *Candida* isolates were collected over a seven-year period (1996–2002) and minimum inhibitory concentrations (MICs) were determined by *E*-test AB Biodisk, Sweden, against amphotericin B (AP), fluconazole (FL), itraconazole (IT), and 5-flucytosine (FC). The test was performed on RPMI 1640 medium supplemented with 2% glucose, pH adjusted to 7.0 with 0.165 M morpholinepropanesulfonic acid buffer, according to the manufacturer's instruction. Isolates showing breakpoints of >2 μg/mL for AP, >64 μg/mL for FL, >1 μg/mL for IT, and >32 μg/mL for FC were graded as resistant. Interpretive breakpoints for FL, IT and FC were the same as recommended in NCCLS, M27A document.

Results: The MIC90 (with MIC range) determined at 24 h incubation for each antifungal agents were as follows: *C. albicans* ($n = 111$), AP 0.38 (0.003–0.75), FL 1.5 (0.047–32), IT 0.75 (0.012–32), FC 0.125 (0.008–2); *C. parapsilosis* ($n = 128$), AP 0.5 (0.004–1.5), FL 2 (0.016–12), IT 0.38 (0.002–1.5), FC 0.25 (0.004–32); *C. tropicalis* ($n = 44$), AP 0.5 (0.023–0.75), FL 1.0 (0.25–4), IT 1.5 (0.008–32), and FC 0.25 (0.002–32); *C. krusei* ($n = 13$), AP 0.38 (0.25–1), FL > 64 (24–>64), IT 4 (0.25–4), FC > 32; *C. glabrata* ($n = 12$), AP 0.75 (0.25–1), FL 16 (2–16), IT 32 (1–32), and FC 0.064 (0.012–0.094).

Conclusions: All the *Candida* isolates were susceptible to amphotericin B. Resistance to azoles for *C. albicans*, *C. parapsilosis* and *C. tropicalis* was <12%. Eighty and >50% of the isolates of *C. krusei* were resistant to itraconazole and fluconazole, respectively. The resistance of *C. glabrata* to fluconazole and itraconazole was 8 and 66%, respectively. Antifungal susceptibility by *E*-test can be conveniently incorporated and performed in a hospital-based clinical laboratory.

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P1502 Development of a bioassay for voriconazole using a *Candida albicans* strain lacking major multidrug transporters and the calcineurin A subunit

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Background: Voriconazole (VCZ) is a novel broad-spectrum triazole antifungal with enhanced antifungal properties and high efficacy in the treatment of fungal diseases refractory to conventional antifungal therapy. The introduction of VCZ in clinical use and its potential related toxicity problems

require the monitoring of serum levels on some clinical situations (difficult to treat infections, liver or renal insufficiency).
Objectives: To develop a rapid, economic, reliable and sensitive method for the determination of VCZ serum levels by a bioassay using a *C. albicans* strain with high susceptibility to VCZ.
Methods: A *C. albicans* strain (DSY2621) was constructed lacking major multidrug transporter genes (CDR1, CDR2, CaMDR1, FLU1) and the calcineurin A subunit gene CNA. This strain combined the advantage of having increased susceptibility to VCZ and a lack of VCZ tolerance, thus increasing detection of low VCZ levels and also facilitating a visual detection in VCZ susceptibility assays. For the development of a bioassay, VCZ was added at fixed concentrations in spiked human serum in different agar media containing the strain DSY2621. The agar plates were incubated at 34 °C for 24 h. Intra- and interrun validation were performed by standard methods and the results compared with an established HPLC method.
Results: The optimal conditions found for this bioassay used regular Yeast Nitrogen Base (YNB) agar medium buffered to pH 7.0 with phosphate buffer and containing an inoculum of 1.5×10^5 cells per mL of medium. The method was validated by intra- and inter-run experiments for a concentration range from 0.3 (accuracy intra- and inter-run: 1.6 and 2%; precision intra- and inter-run: 11 and 3.9%) to 7 µg per ml serum (accuracy intra- and inter-run: −7 and −13%; precision intra- and inter-run: 5.5 and 2.6%). This method was found superior to HPLC in accuracy and precision for low VCZ concentration ranges (0.3–1.5 µg per ml; accuracy intra- and inter-run: −30 and 20.6%; precision intra- and inter-run: 42.1 and 13%), but was equivalent for higher concentration ranges.
Conclusion: The VCZ bioassay developed with the strain DSY2621 can be used for determining VCZ serum concentrations, is simple and does not require a more technically demanding equipment such as HPLC.

P1503 Antifungal susceptibility of yeasts responsible for bloodstream infections

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Antifungal susceptibility is essential to guide antimicrobial therapy in episodes of fungemia. Concerns regarding recent development of antifungal resistance have been raised.
Objectives: We determined the antifungal susceptibility pattern of clinical yeasts isolated from bloodstream infections over a two-year period (2000–2001) and compared it with the previous period (1988–99).
Methods: Susceptibility testing was performed using modified microdilution (M-27 A, NCCLS), with RPMI-2% glucose medium. Antifungal drugs tested were amphotericin B (AB), flucytosine (FC), ketoconazole (KZ), fluconazole (FZ), itraconazole (IZ), voriconazole (VZ), and LY-303366 (LY).
Results: Overall, 89 strains from 74 episodes of fungemia were tested. The MIC90 (µg/mL) of species tested was as follows:

Species (*n*)	AB	FC (%I-R*)	KZ	FZ (%SDD-R*)	IZ (%SDD-R*)	VZ	LY
C. albicans (33)	0.5	0.25 (0)	0.01	0.25 (0)	0.06 (6)	0.004	0.01
C. parapsilosis (29)	0.5	0.125 (0)	0.008	1 (0)	0.06 (0)	0.01	4
C. glabrata (8)	1	0.125 (0)	1	32 (38)	0.5 (100)	0.5	0.03
C tropicalis (7)	1	1 (0)	0.008	0.5 (0)	0.06 (0)	0.03	0.06
C. krusei (3)	1	8 (67)	2	64 (100)	0.25 (100)	0.5	0.06
C. neoformans (2)	0.5	2 (0)	0.125	2 (0)	0.125 (0)	0.03	>128
Other** (7)	1	0.5 (0)	1	16 (43)	0.5 (7)	0.5	2
Overall (89)	1	0.5 (2)	0.5	16 (10)	0.25 (18)	0.25	4

*Flucytosine, >4 µg/mL; fluconazole, >8 µg/mL; itraconazole, >0.125 µg/mL.
**2 Blastoschizomyces capitatus, 2 *C. sake*, 1 *C. guilliermondii*, 1 *Zygosaccharomyces* sp., and 1 *Saccharomyces cerevisiae*.

There were no differences in the susceptibility pattern of this study and the previous one (1988–99; poster number 225, 40th ICAAC).
Conclusions: VZ and AB showed a good activity against the yeasts isolated, with an overall MIC90 of 0.25 and 1, respectively. The remaining azoles and FC were very active except for *C. krusei*, *C. glabrata* (FC was active against this species), and some uncommon yeasts. LY showed good activity against the isolates tested with the exception of *C. parapsilosis*, *C. neoformans*, and some uncommon yeasts. Despite widespread use of antifungal therapy in recent years, we were unable to find significant increases in resistance when compared this susceptibility pattern with the previous surveillance study corresponding to the period 1988–99.

P1504 In vitro susceptibility of *Candida* species isolated from cancer patients against some antifungal agents

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Objective: Antifungal drug resistance is becoming a major problem in certain populations, especially in immunosuppressed patients. This study was undertaken to study the resistance of *Candida* species isolated from oropharengeal swabs of cancer patients to ketoconazole (KET), fluconazole (FLU), itraconazole (ITRA), amphotericin B (Amp B), terbinafine (TRB), flucytosine (FCU) in Ankara University, Faculty of Medicine, Department of Medical Oncology.
Material and methods: The most commonly identified species of *Candida* were *C. albicans* (75), followed by *C. tropicalis* (9), *C. glabrata* (8), C. famata (2), *C. krusei* (1), *C. kefyr* (1), *C. guillermondii* (1), respectively. An adapted NCCLS M 27-A method was used to evaluate the activity of KET, FLU, ITRA, Amp B, TRB and FCU. The MICs of the strains were done using RPMI 1640 medium by microdilution method.
Results: There were no *C. albicans* strains resistant to KET, FLU, Amp B and TRB. There were slight differences in the susceptibility patterns of 22 nonalbicans *Candida* spp. isolates. The *C. krusei* strain showed intermediate resistance to FLU (MIC to FLU 31.25 µg/mL). Resistance to ITRA appeared in 14 strains of *C. albicans* (MIC to ITRA > 1 µg/mL), 6 strains of *C. glabrata*, 1 strains of *C. tropicalis* and 1 strains of *C. guillermondi*. Resistance to FLU appeared in four strains of *C. albicans* (MIC to FLU > 32 µg/mL). So among the 97 strains, 22–23% were resistance to ITRA and 4% were resistance to FCU. All strains were found susceptible to Amp B (MIC to AmpB > 4 µg/mL), KET and FCU.
Conclusion: The main conclusion of this study is that the prophylactic therapy planned according to typing and antifungal sensitivity pattern will be contribute to the preventation of invasive fungal infections in immunosuppressive oncology patients.

P1505 In vitro activity of hexetidine on *Candida albicans*

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Objectives: To evaluate the in vitro effect of 0.1% solution Hexetidine on *Candida albicans*. Hexetidine is used as active ingredient of commercially available mouthwashes for oral hygiene purposes. *Candida albicans* is part of normal oral microflora, besides aerobe and anaerobe bacteria. The criteria considered for this assessment were Minimal Inhibitory Concentration (MIC), killing-time and chlamidospores formation.
Materials and methods: One *C. albicans* isolate was considered for this study. For MIC evaluation, broth macrodilution method in Sabouraud broth was used. The tubes were incubated at 37°C by night before reading. For killing-time assessment the yeast was exposed to Hexetidine 0.1% for a defined period of time (30; 45; 60; 90; 120 s) and then alliquots from the suspension were plated onto Sabouraud – dextrose agar and incubated for 48 h at 37°C. 0.05% Hexetidine solution (half the concentration of commercial product) was used to observe the inhibitory effect on chlamidospores formation after short period of time of exposure (30; 54; 60 s). The effect was evaluated on Rice Agar Tween medium, incubated for 48–72 h at 25°C.
Results: MIC value was found to be 1/32 (31 µL/mL solution or 0.003%) from the initial concentration. Killing-time was 45 s and inhibition for Chlamidospores formation was obtained after 45 s exposure at 0.05% Hexetidine.
Conclusions: Our results confirmed the usefulness of Hexetidine for oral purposes if used correctly, according to the manufacturers recommendation for at least 45 s mouth rinse.

P1506 In vitro interaction between flucytosine and other antifungals against *Cryptococcus neoformans*

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Objectives: to evaluate the in vitro interaction of flucytosine (5FC) in combination with azoles or amphotericin B (AMB) against clinical isolates of *Cryptococcus neoformans*.

Methods: a NCCLS M-27 A microdilution broth technique modified for checkerboard studies was used. Two-drug combinations of 5FC with fluconazole (FCZ), itraconazole (ITZ), voriconazole (VRZ), or AMB were evaluated against 30 recent clinical isolates of *C. neoformans*. After 72 h of incubation microplates were read spectrophotometrically and MICs were defined as the lowest concentration that gave 50% (azoles and 5FC) or 95% (AMB) of inhibition. FIC indices (FICI) were calculated and interactions were classified as synergistic (FICI ≤ 0.5), additive (FICI > 0.5 and ≤ 1), indifferent (FICI > 1 and ≤ 4), and antagonistic (FICI > 4). All experiments were run in duplicate.
Results: (see Table)

	% of stains			
Interaction	5FC + FCZ	5FC + ITZ*	5FC + VRZ	5FC + AMB
Synergistic	77	60	80	77
Additive	20	32	20	23
Indifferent	3	8	0	0
Antagonistic	0	0	0	0

*Tested on 25 strains

Geometric mean MICs (range) for the drugs alone were 4 (0.5–64), 1.95 (0.25–8), 0.11 (0.03–0.5), 0.02 (0.004–0.125), and 0.62 μg/mL (0.25–1) for 5FC, FCZ, ITZ, VRZ, and AMB, respectively. Even with some isolates exhibiting high MICs for 5FC, combination with AMB or azoles was synergistic.
Conclusions: combination of azoles with 5FC showed similar synergistic interaction compared with the combination of AMB with 5FC. Antagonism was not observed.

P1507 Use of the Sensititre Colorimetric Microdilution Panel for antifungal susceptibility testing of dermatophytes

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Objective: Compare the susceptibility of different dermatophytes species to itraconazole (I), voriconazole (V) and fluconazole (F) by the reference method (MD) (NCCLS, M38A) and the colorimetric method Sensititre Yeast One (R).
Material and methods: We studied a global of 47 strains of dermatophytes (30 *T. mentagrophytes*, 9 *T. rubrum*, 8 *M. gypseum*) isolated from clinical specimens. The MD reference susceptibillity testing was performed following the NCCLS M38A document, using drug dilutions from 0.03 to 16 mg/L for I and V, and 0.125–256 mg/L for F. The MICs were defined as the lowest concentration that produced the 50% inhibition of the growth, we read after 4–6 days of incubation at 35°C. The Sensititre Yeast One (R) was performed following the manufacturer's instructions, and the inoculum used was the same that for the MD. The MICs were read in the well where the colour change from pink to blue or purple was observed. Quality control strains: *C. parapsilopsis* ATCC 22019 and *C. krusei* ATCC 6258.
Results: The levels of agreement between the two different methods (±2 dilutions) were for *T. mentagrophytes* 30, 53.3 and 83.3%; *T. rubrum* 0, 12.5 and 66.6%; *M. gypseum* 37.5, 44.4 and 75% for for I, F and V, respectively. The MICs 50/90 (mg/L) for the different species were: *T. mentagrophytes* 0.25/0.5 (I), 0.12/0.25 (V), 16/64 (F) (MD) and 0.016/0.06 (I), 0.03/0.06 (V), 8/16 (F) (Sensititre). *T. rubrum* 0.25/1 (I), 0.25/0.5 (V), 8/64 (F) (MD) and 0.008/0.03 (I), 0.016/0.03 (V), 2/8 (F) (Sensititre). *M. gypseum* 0.5/1 (I), 0.25/1 (V), 64/256 (F) and 0.016/0.25 (I), 0.06/0.25 (V), 16/256 (F) (Sensititre).
Conclusions: (i) The MICs obtained were lower by Sensititre than by MD. (ii) The best correlation between both methods was obtained for F and *T. mentagrophytes* (>80%). (iii) *T. rubrum* showed a very low correlation, perhaps due to the difficulty to obtain standardized inocula because it has low levels of sporulation or high levels of pleomorphism. (iv) Although Sensititre is an easy method which could be used in a clinical laboratory, it shows poor agreement with the reference method for dermatophytes, however, more studies are necessary.

P1508 Isolation of griseofulvin-resistant strains of dermatophytes from Isfahan

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Objective: The emerge of drug resistance in dermatophytes would affect the incidence of infection in the society and causes difficulties for both physician and patient. With report of cases of griseofulvin resistant dermatophytes, the use of new antifungal drugs is recommended which are more expensive or somehow rare in Iran. Therefore, the necessity of griseofulvin sensitivity pattern of dermatophytes in Isfahan is perceived which could lead to a more effective and less expensive treatment for dermatophytoses.
Methods: Fifty isolates of the most prevalent dermatophytes in Isfahan were isolated from patients and then the standard homogenized suspension from them were prepared for future inoculation. The minimum inhibitory concentration (MIC) of griseofulvin was determined by modified macrodilution method for each isolate and then results were compared and analyzed with standard values of MICs of dermatophytes and resistant strains identified.
Results: All 100% tested isolates had MIC mode of <0.25, 90% had <8 and 50% ranged between <0.25–1 μg/mL. From all isolates, 10% of them including three *Trichophyton verrucosum*, one *Microsporum canis* and one *T. mentagrophytes* had MIC out of standardized range therefore, they considered as griseofulvin resistant isolates.
Conclusion: Although MIC values of drugs at in vivo and in vitro are somewhat different but in vitro values could be used as additional parameters in the decision making of treatment for dermatophytoses, in particular its recalcitrant types or in areas which the resistant species may have high prevalence.

Nosocomial nonfermenting Gram-negative bacterial infections: clinical and laboratory studies

P1509 Outbreak of hospital-acquired monoclonal *Pseudomonas aeruginosa* infection associated with the use of contaminated commercial mouth swabs in a primary and a tertiary care hospital in Norway

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Objective: We present differences in outbreak epidemiology of hospital-acquired monoclonal *P. aeruginosa* outbreak data from a Norwegian primary care (PCH), Baerum Sykehus, and a tertiary care hospital (TCH), Rikshospitalet. The data are part of a national hospital-acquired outbreak associated with the use of contaminated commercial mouth swabs.
Methods: During the study period from December 2001 to June 2002 isolates of *P. aeruginosa* were stored for later analysis. Pulsed-field gel electrophoresis was used for strain identification.
Results: From ultimo December 2001 to ultimo June 2002 the strain was isolated from 12 patients in the 250-bed PCH and 6 patients in the 585-bed TCH. In the majority of the patients, the strain was isolated from various airway secretions (10 patients in the PCH, 5 in the TCH). Most patients were infected in the ICUs (seven and four of the PCH and TCH patients, respectively), while the rest were infected in various medical wards. Three of the patients died of *P. aeruginosa*-associated multiorgan failure within 2 days of positive blood culture. Four other patients died during the study period, *P. aeruginosa* infection may have contributed. In the PCH, the outbreak started in December 2001 and lasted until April 2002, while in the TCH the outbreak started in March 2002 and lasted until June 2002. In February 2002 the Norwegian Institute of Public Health alerted of increasing incidence of *Pseudomonas* infection in several hospitals. On 9 April 2002, commercially available mouth swabs were identified as the source of the outbreak. Later, it was recognized that the product was contaminated during the packing process by the manufacturer. Culture of sputum thrice weekly was part of standard ICU screening in the TCH, none in the PCH.
Conclusion: The reason for the lower incidence of *P. aeruginosa* infection in the TCH and the later peak incidence is unknown. However, different use of mouth swabs as well as random variations in the use of contaminated batches may have contributed. Various practice by the laboratory personnel in

collecting *P. aeruginosa* strains may also explain differences. Sputum screening seems not to have contributed significantly. Last but not least, empiric antibiotic treatment with meropenem may have limited the outbreak in the ICU of the TCH compared with the PCH in which cephalosporins were used more widely.

P1510 Nosocomial infections due *to Xanthomonas* (*Stenotrophomonas*) *maltophilia*: an epidemiologic and microbiologic surveillance

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Introduction: *Xanthomonas* (*Stenotrophomonas*) *maltophilia* is increasingly recognized as an important cause of nosocomial infection. Infection primarily, but not exclusively, affects immunocompromized patients. Although this organism has been considered to have limited pathogenicity, reports indicate that infection with *S. maltophilia* can cause bacteremia and other serious infection, e.g. bronchopenumonial and catheter-related infections.
Methods: We performed a surveillance about this Gram-negative bacterium in our hospital (Pescara, Italy) during the years 2001–2002. We isolated 45 strains in patients admitted to ICU, medical and surgical departments. Microbiological examinations of specimens were performed with McConkei agar and antimicrobial test with Kirby–Bauer method, according to NCCLS standard.
Results: Thirty-two out of 45 strains of *S. maltophilia* were isolated in ICU, 8 out of 45 in surgical departments and 5 out of 45 in medical departments. Forty-six percent of strains were susceptible to amikacin, 28% to cefotaxim, 75% to ceftazidim, 10% to ceftriaxone and imipenem 75% trimethoprim–sulfamethoxazole, 28% amoxicillin/clavulanate.
Conclusions: Management of *S. maltophilia* associated infections is problematic because many strains of the bacterium are frequently resistant to multiple antibiotics, including those of the carbapenem class. In our experience, risk factors for *S. maltophilia* infection include neutropenia, the presence of CVC, prolonged hospitalization, and previous therapy with antibiotics. Despite its acknowledged importance as nosocomial pathogen, little is known of the epidemiology of *S. maltophilia*, and its reservoirs are often not apparent.

P1511 Adhesion of *Acinetobacter* spp. to *para*-xylene

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Bydgoszcz, PL

Objective: The objective was measuring adhesion of 200 *Acinetobacter* spp. strains to para-xylene describing surface hydrophobicity bacterial cells.
Methods: Hydrophobicity was defined by the Bacterial Adhesion to Hydrocarbons Test (BATH) method of Rosenberg.
Results: Forty-eight percent strains of *Acinetobacter* spp. adhered to *para*-xylene. Forty percent of strains showed weak hydrophobicity, 7.5% mild and 1 strain strongly adhered to *para*-xylene. *A. baumannii* strains adhered to polymers in 52.2%, *A. junii* in 42.1%, and 20.0% in *A. haemolyticus*. BATH test is useful technique for study hydrophobic properties of many bacteria surfaces attached to polymers.
Conclusions: About half strains of *Acinetobacter* spp. showed hydrophobic properties. Significantly we find weak adhesion to *para*-xylene than mild and strong. Mostly adhesion was discovered in *A. baumannii* and in *A. junii* strains than in *A. haemolyticus*.

P1512 Adhesion of *Acinetobacter* spp. to polystyrene

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Objectives: The objective was estimation adhesive properties Gram-negative rods *Acinetobacter* spp. to polystyrene.
Methods: Adhesion was measured by the method Christensen using polystyrene 96-well, flat-bottomed tissue culture plates which were filled diluted cultures of bacteria in trypcase soy broth (TSB) and in 0.5 TSB. Tissue culture plates were incubated for 18 h at 37 °C, washed four times and stained with crystal violet. Adhesion was measured by optical density (OD) of stained bacteria to polystyrene plates.
Results: Among 200 strains of *Acinetobacter* spp. 72.5% adhered to polystyrene in TSB and 68.0% in 0.5 TSB. We discovered adhesive properties in *A. baumannii* strains ($n = 157$) in 77.1% when bacteria were incubated in TSB and in 71.3% in 0.5 TSB. *A. junii* ($n = 19$) showed adherence to polystyrene in 57.9% in TSB and in 0.5 TSB. *A. haemolyticus* ($n = 15$) adhered in 60.0% in TSB and in 53.3% in 0.5 TSB. Significantly we find strong adhesive properties when *A. baumannii* were incubated in TSB compared with 0.5 TSB.
Conclusions: An optical density of stained bacteria to plastic tissue culture plates is useful method for study bacterial adherence to medical devices. Most of all strains of *Acinetobacter* spp. adhered to polystyrene. Most often strong adhesion to polystyrene showed bacteria incubated in TSB than in 0.5 TSB.

P1513 Clinical outcomes and effectiveness of antimicrobial treatment of patients with antibiotic-resistant *Pseudomonas aeruginosa* pneumonia

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Objective: To evaluate the clinical outcomes, length of stay and antibimicrobial treatment of patients with antibiotic-resistant *Pseudomonas aeruginosa* pneumonia.
Methods: Data were analyzed from histories of 102 patients (pts) with positive clinical cultures for *P. aeruginosa* who were treated in ICU of Kaunas Medical University Hospital. In-hospital mortality and length of stay were examined in 66 pts who had *P. aeruginosa* resistant to imipenem, meropenem, ceftazidime or amikacin and compared with the rest of 36 pts that had *P. aeruginosa* susceptible to the earlier mentioned antibiotics. The overall in-hospital mortality in ICU during the year 2000 was 15.47%, the mortality in pts with susceptible *P. aeruginosa* was 38.88% vs. to 63.63% of pts from whom resistant *P. aeruginosa* was isolated (relative risk [RR], 1.64; 95% confidence interval [CI], 1.087–2.645). The duration of hospital stay for pts with a susceptible *P. aeruginosa* was 9 (8.6 ± 1.2) days vs. 19 (19.4 ± 2.3) days for pts who had resistant *P. aeruginosa* isolated ($P < 0.001$). In pts group with resistant *P. aeruginosa* ($n = 66$) treatment with I, M, CF and A was more effective than that with other antipseudomonal antibiotics (mortality 53.3% vs. 85.7%, $P < 0.05$). Relative risk reduction (RRR) −0.378, 95% [CI] (0.118–0.557), number of patients who need to be treated (NNT) to prevent one death – 3.09, 95% [CI] (1970–12 597), incremental cost per death avoided (ICER) – 1143 €. Descending (strong – weak) empiric treatment was more effective than ascending (mortality 30% vs. 52.43%, $P = 0.08$), relative risk reduction (RRR) – 0.428, 95% [CI] (− 0.042–0729), number of patients who need to be treated (NNT) to prevent one death – 4457 95% [CI] (2383 to infinity), incremental cost per death avoided (ICER) – 1194.05 €.
Conclusion: Infections caused by antibiotic-resistant *P. aeruginosa* bacteria result in higher mortality and prolonged hospital stay. Treatment with I, M, CF, and A was more effective than that with weak antipseudomonal activity antibiotics.

P1514 Hydrophobicity of *Pseudomonas aeruginosa* clinical isolates: comparison 1991–2002

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Porto, P

Introduction: The versatility of opportunistic bacteria is due to the fact that different sets of strains can acquire different groups of virulence genes, without species barrier. Bacterial adhesion to biotic or abiotic surfaces, as the use of medical indwelling devices is more widespread, is the first step of bacterial pathogenicity. Adherence to liquid hydrocarbons is an indirect method to assess microbial cell surface hydrophobicity (CSH), which is believed to have an important role in the adherence of bacteria.
Objective: To determine the correlation between hydrophobicity with origin products and to compare distributions in the same general hospital in 1991 and 2002
Materials and methods: Fifty random selected clinical isolates of *P. aeruginosa* from different biological products (respiratory tract, SB; Urine, U; and pus, P were assayed in percentage (in an assay for hydrocarbon adhesion (1)) for the capacity to adhere to *n*-hexadecane to assess hydrophobicity. All tests were run in triplicate.
Results: The values are expressed in percentage as mean ± standard deviation: SB, 1991: 17 ± 16; 2002: 23 ± 17; U 1991: 31 ± 29; 2002: 20 ± 15; P 1991: 55 ± 23; 2002: 14 ± 5.
Conclusions: Considering that the variation is due to the source of the isolate an analysis of variance (ANOVA) results in a significant difference ($P < 0.01$) between adhesion values to epithelia (SB and U) than to no epithelial surfaces

(P) in 1991. These results were not found in 2002. This can me explained by the constant exchange of pathogenicity islands of genes between strains of nosocomial origin, namely genes related to the expression of hydrophobins or pili.

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Reference

1. Van der Mei *et al. Curr. Microbiol* 1991, 23, 337–41.

P1515 Serotype and adhesion to biomaterials *Pseudomonas aeruginosa* in a general hospital, Portugal

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Porto, P

Introduction: *Pseudomonas aeruginosa* has been reported to colonize epithelia and indwelling medical devices. Lipopolyssacarides (LPS) could be involved in promoting adhesion to biomaterials.

Objectives: To study *Pseudomonas aeruginosa* epidemiology in a Central Hospital in Portugal and to compare bacterial surface hydrophobicity in two periods (1991 and 2002).

Materials and methods: Fifty randomly selected strains isolated from different biological products were used in this study. The bacterial serotypes were typed with *P aeruginosa* antisera. The attachment of bacteria to biomaterials was assayed in percentage for the capacity to adhere to *n*-hexadecane to assess hydrophobicity (1). Three different classes of hydrophobicity were obtained ($>0.4 = 2$; $<0.1 = 0$; >0.1 and $<0.4 = 1$). All tests were run in triplicate.

Results: Frequencies of the serotypes in 1991 and 2002: O:1, 13%, 20%; O:2, 16%, 1%; O:3, 9%, 4%; O:4, 6%, 1%; O:5, 4%, 1%; O:6, 21%, 26%; O:7, 3%, 6%, O:8, 8%, 4%; O:9, 1%, 4%; O:10, 23%, 3%; O:11, 16%, 11%; O:12, 17%, 4%; O:13, 10%, 0%; O:14, 2%, 4%; O:15, 2%, 10%; O:16, 6%, 3%. Serotypes O:6, O:1, O:11 have higher hydrophobicity values and frequencies in 1991 and 2002, O: 1O had lower frequency but similar hydrophobicity comparing 1991 and 2002.

Conclusions:

1. Serotypes O:6, O:1, and O:11 are the ones with higher frequencies and hydrophobicity.
2. The results indicate that the surface characteristics of bacterial serotypes are related to the bacterial adhesion to the surface, but the pathogenesis of the bacteria surely results from multiple factors.

Acknowledgements: This study was supported by a research grant from Fundação Calouste Gulbenkian.

Reference

1. Van der Mei *et al.* 1991 *Curr. Microbiol;* 23: 337–41.

P1516 Adherence ability to abiotic surfaces in opportunistic *Pseudomonas aeruginosa*: a correlation study

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Introduction: Attachment to biotic or abiotic surfaces is the first step of bacterial pathogenicity. There is a need to study how prevalent is this ability to adhere and create sessile communities in nosocomial bacteria.

Objectives: The aim of the present study was to investigate the correlation between *Pseudomonas aeruginosa* adherence potential to different substrata and to access the involvement of acid–base (AB) interactions in microbial adhesion.

Materials and methods: One hundred randomly selected strains of opportunistic *Pseudomonas aeruginosa*, from different sources were screened for their adhesion capacity to hydrophobic biomaterials such as silicone, hexadecane, and to electron–accepting chloroform, as assayed previously (1). All tests were run in triplicate.

Results: There was a significant correlation (Spearman correlation coefficient) between silicone and hexadecane (0.67; $P = 0001$), between silicone and chloroform (0.29; $P = 0004$). The mean ± standard deviation adhesion values are: to silicone (0.15 ± 0.08), to hexadecane (0.21 ± 0.14), and to chloroform (0.50 ± 0.14).

Conclusions: Adhesion to hydrophobic *n*-hexadecane can be used for measuring the potential ability to attach to biomaterials because it correlates well with silicone although it underestimates hydrophobicity. In fact, acid–base (AB) interactions are absent in *n*-hexadecane and present in the adhesion to chloroform, which explains higher adhesion values to this surface (2). Hydrophobicity assays for large numbers of strains should always involve a comparison between adhesion to *n*-hexadecane and adhesion to chloroform in order to understand physicochemistry involved in microbial adhesion.

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P1517 Prevalence of swimming, swarming and twitching in clinical isolates of *Pseudomonas aeruginosa*: correlation to the adherence capabilities to biomaterials

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Introduction: *Pseudomonas aeruginosa* is a human opportunistic pathogen able to organize itself in very structured communities called biofilm, which allows the bacterium to colonize various environments and infectious sites. Biofilm formation represents an important aspect of the pathogenic power of this microorganism. Flagellum and type IV pili are known to be involved in the first step of biofilm formation (1), so it is important to understand their prevalence among clinical isolates.

Objectives: To study the prevalence of flagella (swimming), flagella and pili (swarming) and pili (twitching) in a random selected set of nosocomial strains and to correlate to their adhesion capabilities to abiotic surfaces.

Materials and methods: One hundred randomly selected strains from different origins of opportunistic *Pseudomonas aeruginosa* were screened for their capacity to swim (presence of flagella), swarm (presence of flagella and pili type IV) and twitching (pili type IV) as previously described (1, 2). The percentage of adhesion potential to *n*-hexadecane was obtained as assayed previously (3) and afterward transformed in a ordinal variable for statistical analysis. All tests were run in triplicate.

Results: Swimming (58% with flagella only; 42% without); swarming (35% with flagella and pili type IV; 65% without): Twitching (17% with pili type IV only; 83% without). There was no significant relation between swimming and hydrophobicity and between swarming and hydrophobicity but there was a significant relation between twitching and hydrophobicity (Pearson Chi-square 6.3: d.f. 2: $P = 0043$).

Conclusions: Surface approach is done by flagella, which justifies higher prevalence comparing to the presence of pili type IV or both, required for surface translocation. The presence of pili type IV increases surface hydrophobicity which promotes higher adhesion potential to biotic or abiotic surfaces.

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P1518 The effect of simultaneous application of therapeutic ultrasound and ceftazidime on growth of *Pseudomonas aeruginosa*

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Objectives: *Pseudomonas aeruginosa* is an important human opportunistic pathogen with innate resistance to many antibiotics and disinfectants, predominantly infecting patients with defects in antibacterial host defenses. Ultrasound is currently used in medical practice for diagnostic and therapeutic purposes. A recent and relatively new application of ultrasound is in drug delivery. There are many reports in the literature suggesting that ultrasound activates, potentializes or makes more effective some pharmacological agents. In the present study, we investigated the lethal effect of ultrasound and sMICs of ceftazidime on growth of *Pseudomonas aeruginosa* (ATCC 27853) separately and in combination.

Methods: The MIC of ceftazidime for this strain was determined in Mueller–Hinton Broth by the macrodilution technique. The aqueous suspension of microorganism in a sterile, sealed plate was placed in an ultrasonic tank operating at 1 MHz. Different power output were used: 0.25, 0.5, 1 and

1.5 W. After desired time of exposure to the ultrasound (30 and 60 s), each sample was plated separately and after incubation, the number of colonies was counted.
Results: Bactericidal effect of ceftazidime concentration equal to 1 μg/mL against this bacterium was more than that observed with 0.5 μg/mL statistical analysis of results showed that there was a significant effect of intensity for this bacterium, with percent killed increasing with increased intensity level and a significant effect of time, with percent killed increasing with increased duration of exposure. The combination of ultrasound and sMICs of ceftazidime was much more lethal to this bacterium than either of the treatments alone.
Conclusion: These data confirmed that simultaneous application of ultrasound and ceftazidime has some efficacy in inactivating *Pseudomonas aeruginosa*. The physical mechanism of inactivation by ultrasound alone, appears to be transient cavitation, but the mechanism of synergistic effect of ultrasound and ceftazidime is unknown. It might be a result of an enhanced penetration of the antibiotic through the outer membrane. This promising effect may results in developing a new methodology of killing resistant bacterial infections.

P1519 Non-fermenting Gram-negative bacilli other than *Pseudomonas aeruginosa* from clinical specimens

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Objectives: To identify nonfermenting Gram-negative bacilli (NFB) other than *Pseudomonas aeruginosa* and to determine antimicrobial susceptibility, clinical relevance and response to therapy.
Methods: The NFB isolated from outpatients and inpatients seen at the Amiri Hospital, Kuwait from September to December 2002 were identified by conventional methods, the API 20NE and/or Vitek 2. Antimicrobial susceptibility testing was performed by the modified Kirby–Bauer method and where applicable the Vitek 2. Clinical relevance was assessed from the condition of the patient, source of the specimen and response to therapy.
Results: A total of 88 NFB were isolated from 87 patients. Twenty-six isolates were from urine, 26 from skin and soft tissues, 24 from the lower respiratory tract, 7 from peritoneal dialysis fluids, and 5 from blood. Of the total, 47 were hospital acquired. Thirty-two hospital acquired and eight community-acquired infections were significant. Clinically significant hospital infections were associated with the use of ventilators, catheters or intravenous lines. Forty-seven isolates were *Acinetobacter* spp., 16 *Stenotrophomonas maltophilia*, 6 Alcaligenes spp., 4 *Comamonas* spp. and 15 isolates from 9 other genera. Isolates from hospital acquired infections were more resistant to antibiotics compared with community acquired isolates. Multiple antibiotic resistant *Acinetobacter* spp. were isolated from two patients. Significant infections responded to therapy except in three cases.
Conclusions: NFB may be isolated from a wide variety of clinical specimens. Although they are often encountered as colonizers, significant hospital acquired infections are common. Their accurate identification and susceptibility testing may ensure appropriate treatment.

P1520 Typing of clinical isolates of *Stenotrophomonas maltophilia* strains by pulsed-field gel electrophoresis

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Objective: To determine the clonally profiles of clinical strains of *S. maltophilia* isolated during a 1-year period.
Methods: During a 1-year study period 128 strains of *S. maltophilia* were isolated from different specimens of 76 patients at a 1500-bed major tertiary care teaching university hospital. The strains were identified by commercial available API system and genotyped by pulsed-field gel electrophoresis (PFGE) using the CHEF-DR III system. The restriction enzyme was *Spe*I.
Results: The PFGE profiles of the clinical isolates of *S. maltophilia* strains varied widely. Only few isolates from different specimens of the same patient were clonally identical. In other patients the strains of the same patient isolated from different specimens were clonally different.
Conclusion: Due to the wide variation of their PFGE profiles, our results suggest that there was no outbreak situation of *S. maltophilia* strains with identical profiles at our hospital.

P1521 Study of the evolution of multiresistant *Acinetobacter baumannii* clones isolated in a hospital in northern Spain

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Leioa, E

Objectives: The aim of this study was to analyze the evolution of *A. baumannii* multiresistant clones responsible of nosocomial infections isolated at the Hospital de Sta. Marina (northern Spain) and the evolution of class 1 integrons.
Methods: The study included all isolates obtained at the Microbiology Service during the years 1999 and 2002 (a total of 102 and 82 isolates, respectively). Susceptibility to antimicrobial agents was determined by the agar dilution method following the NCCLS recommendations. Total DNA was used as target for PCR-fingerprinting experiments with primers M13 and ERIC2. To detect class 1 integrons primers 3′CS and 5′CS were used in amplification experiments.
Results: The same three main clones identified in 1999 (named I–III) are still the most important in 2002. However, as clone II was the predominant in 1999, at present the majority of isolates belong to clone I. Concerning the antibiotic resistance, all clones showed an important increase on resistance to amikacin. Moreover, clone I showed a general increase to the resistance to all antibiotics tested specially imipenem and meropenem. The integrons detected in 2002 were the same than the ones identified in 1999 (named a, b and e) with the difference that isolates from the year 2002 bored combinations of at least two bands.
Conclusions: The majority of multiresistant *A. baumannii* isolates in Hospital de Sta. Marina belonged to clone II in 1999 and to clone I in 2002. The same class 1 integrons were detected but the isolates obtained in 2002 belonging to clone I, bored combinations of two or three bands probably related to the increase of the resistance.

P1522 *Achromobacter* (*Alcaligenes*) *xylosoxidans* in a Greek cystic fibrosis unit

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Objective: This study was performed to assess the prevalence of *Achromobacter xylosoxidans* among Greek Cystic fibrosis (CF) patients, to determine the sensitivity of isolated strains to antibiotics, to investigate whether these patients share common genotypes, and whether they persistently carry the same *A. xylosoxidans* strain or they acquire a different one.
Methods: *A. xylosoxidans* isolates that were recovered during 1 year from CF patients were tested. Quantitative cultures of the sputum were processed to appropriate media. Isolates were identified to the species level by the API 20 NE and Wider systems. MICs were determined by the Wider system and *E*-test. They were tested by PCR for the carbapenemase encoding genes blaIMP and blaVIM and genotyped by pulsed-field gel electrophoresis (PFGE).
Results: Samples from eight of 70 CF patients (11.4%) yielded 32 *A xylosoxidans* isolates during the study period. In all cases but one (who remained in a good clinical condition) *Pseudomonas aeruginosa* was concomitantly isolated. All strains were resistant to aztreonam, tobramycin, gentamicin, all but one resistant to amikacin and cefepime, 90.6% to ampicillin/sulbactam, 81.2% to ciprofloxacin, 50.0% to ticarcillin/clavulanic acid, 43.7% to colistin, 40.6% to imipenem and meropenem, and 34.4% to ceftazidime. The most active antibacterial agents were piperacillin (81.2% sensitive) and its combination with tazobactam (90.6% sensitive). Genes blaIMP and blaVIM were not detected in the carbapenem-resistant isolates. Strains that were recovered from most patients belonged to the same genotype, while the same strain persisted in each patient during the study period.
Conclusions: The prevalence of respiratory isolation of *A. xylosoxidans* among our CF patients was higher than that reported previously. Piperacillin and its combination with tazobactam remained effective against most of these *A. xylosoxidans* isolates, while colistin was active against only 56.3% of isolates. The high rate of resistance to carbapenems was not due to the production of known carbapenemases. The recovery of genetically related isolates from most patients indicates that *A. xylosoxidans* was acquired from an unknown common source and infected CF lungs in a persistent way.

P1523 An outbreak of multidrug-resistant strains of *Acinetobacter* spp. in an Iranian hospital during 2001–2002

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Objective: The aim of this study was to determine antimicrobial susceptibility of hospital acquired isolates of *Acinetobater* spp. in Milad Hospital of Tehran.
Methods: From 1st July 2001–30th November 110 strains of *Acinetobacter* spp. isolated from urine, blood, wound and other clinical specimens of hospitalized patients. All isolates identified according routine microbiological methods. The antimicrobial susceptibility testing were performed by disk diffusion method according NCCLS M100-S12 guideline.
Results: Of 110 strains of *Acinetobacter* 58 strain (52.75) isolated from urine, 32 strain (29%) isolated from wound, fluids and 20 strain (18.2%) isolated from blood. More than 80% of strains isolated from patients hospitalized in ICU, NICU, RCU and pediatric department. All isolated strains were resistant to ceftazidime, ceftizoxime, cephalotin, ceftriaxon, cefixim cfazoline and ampicilln. Frequency of resistance to ciprofloxacin, gentamycin, amikacin, trimethoprime–sulfamethoxazol and ofloxacin was 65, 76.5, 85.2, 85.7, and 87.5%, respectively.
Conclusion: Our study showed a high rate isolation of multidrug-resistant *Acinetobacter* spp. from clinical specimens in this hospital. To prevent spread of multidrug-resistant strains of *Acinetobacter*, it is recommended an implementation of control measures and the rational use of antibiotics.

Gram-negative resistance

P1524 Resistance evolution and extended-spectrum-beta-lactamase producing *E. coli* isolated during 4 years

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Objective: To study the resistance evolution and ESBL producing *E. coli* strains isolated in outpatients during the last 4 years.
Methods: Eight thousand nine hundred and seventy-six *E. coli* strains were studied in outpatients from January 1999 to October 2002. The identification and susceptibility were performed by the Walkaway MicroScam System (DADE-Behring). Screening for detection of ESBL-producing strains were: ceftazidime and/or cefotaxime and/or aztreonam more than 1 μg/mL. The detection was performed by the double disk synergye test.
Results: The number of *E. Coli* isolated were: 2666 in 1999, 2234 in 2000, 2586 in 2001 and 1890 in 2002. The number of ESBL-producing *E. Coli* were: 30 (13.2%) in 1999, 47 (2.10%) in 2000, 21 (0.81%) in 2001 and 30 (1.58%) in 2002. The susceptibility each year was: Amikacin was: 98, 99, 98 and 99%. Augmentin 89, 90, 89 and 89%. Ampicillin 41, 42, 42 and 41%. Aztreonam: 93, 87, 83 and 85% Cefotaxime: 97, 99, 97 and 96%. Ceftazidime: 97, 98, 97 and 96%. Imipenem: 98, 99, 99 and 99%. Ciprofloxacin: 81, 84, 83 and 83%. Ofloxacin: 82, 84, 84 and 82%. Fosfomicin: 97, 97, 96 and 97%. Gentamicin: 91, 92, 92 and 92%. Piperacillin: 44, 44, 43 and 42%. T/S: 70, 72, 72 and 72%. The susceptibility of ESBL-producing *E. Coli* were: Ak: 97, 96, 95 and 97%. Aug: 67, 66, 57 and 57%. Im: 100, 100, 95 and 97%. Cp: 80, 70, 43 and 67%. Ofl: 77, 66, 43 and 53%. Fo: 85, 98, 95 and 90%. Gm: 90, 97, 81 and 100%. Pi: 53, 30, 38 and 23%. T/S: 73, 64, 71 and 57%.
Conclusions: The frecuency of ESBL-producing *E. coli* strains is similar during the 4 years studied. The resistance to aminoglycosides, augmentin, cephalosporines, quinolones, fosfomicin and T/S is similar along the time. The ESBL-producing *E. coli* are more resistant to quinolones and augmentin than non-ESBL producing.

P1525 Antimicrobial resistance of Enterobacteriaceae isolated in 2002

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Objective: To evaluate the prevalence of antimicrobial resistance and of extended-spectrum beta lactamases (ESBL) in Enterobacteriaceae isolated in 2002.
Methods: A total of 1515 isolates of Enterobacteriaceae (702 *E. coli*, 265 *K. pneumoniae*, 42 *K. oxytoca*, 87 *Proteus* spp., 79 *Enterobacter* spp., 15 *Citrobacter* spp., 140 *Shigella* spp., 180 *Salmonella* spp.) were isolated from blood, urine, stool, lower respiratory tract and another clinical specimens. The resistance was evaluated by disk diffusion method according to the NCCLS quidelines. Double disk diffusion method and *E*-test were performed for the detection of ESBL producers.
Results: The resistance rate to ampicillin for the majority of species was between 40 and 89.8%. To amoxicillin/clavulanic acid the prevalence of resistance varied: 7.1% in *K. oxytoca*, 12.3% in *E. coli*, 19.5% in *Proteus* spp. and 41.5% in *K. pneumoniae*. Only *K. pneumoniae* and *Enterobacter* spp. were highly resistant to cephalosporins, 26.7 and 35.4% respectively. All Enterobacteriaceae strains tested were susceptible to imipenem. For fluoroquinolones the resistance rate was higher for *K. pneumoniae* (19.2%) and *E. coli* (15.5%). Resistance of *Shigella flexneri* and *Shigella sonnei* were, respectively: ampicillin 77.8 to 21.1%, trimethoprim/sulfamethoxazol 62 to 72.6% and nalidixic acid all susceptible to 18.9%. Non typhoidal *Salmonella* isolated in stool cultures which belongs to serogroup D (70%) and B (20.5%) were resistant 23.8% to nalidixic acid, 8.3% to ampicillin and 5% to trimethoprim/sulfamethoxazol. There was no resistant strain of *Shigella* spp. and *Salmonella* spp. to fluoroquinolones. There are some differences in the rate of resistance for *E. coli* from urinary tract infections in outpatients comparatively with those from inpatients: 11.3%, respectively, 19.6% for ciprofloxacin and 2.5–6.1% for cephalosporins. *K. pneumoniae* recovered from urine was more resistant to almost antimicrobial agents than isolates from respiratory tract infections. The rates of ESBL producers was 26.2% in *K. pneumoniae*, 9.5% in *K. oxytoca*, 5.3% in *E. coli* and 4.5% in *Proteus* spp.
Conclusions: High level of resistance was seen for ampicillin, amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazol against majority of species. The imipenem remain the most potent agent tested against Enterobacteriaceae isolated in our laboratory. ESBL producer's strains were between 4.5% in *Proteus* spp. and 26.2% in *K. pneumoniae*.

P1526 Resistance of Enterobacteriaceae from tracheal aspirates to beta-lactaman and ciprofloxacin in an emergency hospital, Bucharest, between 2001 and 2002

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Objective: To assess the resistance to beta- lactam and ciprofloxacin of clinically relevant Enterobacteriaceae isolated in tracheal aspirates between 2001 and 2002, in Emergency Hospital, Romania
Methods: During the study the resistance of 234 Enterobacteriaceae isolated from tracheal aspirates in ICUs was tested by MIC – 'Microscan walkway – Dade Behring' to seven antibiotics: amoxicillin/clavulanic acid (amc), cefoperazone (cfp), cefotaxime (ctx), ceftriaxone (cro) ciprofloxacin (cip), imipenem (ipm), piperacillin/tazobactam (tzp).
Results: The distribution of bacterial species was as follows: *Klebsiella pneumoniae* 40.4%, *Proteus mirabilis* 22.6%, *E. coli* 18.3%, *Enterobacter aerogenes* 6.85%, *Citrobacter freundii* 5.95% and *Seratia marcescens* 5.5%. The resistance of the Enterobacteriaceae isolates to beta -lactams and ciprofloxacin was: *Klebsiella pneumoniae* (amc 66%, cfp 92.8%, ctx 100%, cro 100%, cip 78.5%, ipm 18.2% tzp 51%). *Proteus mirabilis* (amc 85.2%, cfp 93.3%, ctx 100%, cro 100%, cip 78.5%, ipm 17.5%, tzp 51%). *E. coli* (amc 30.5%, cfp 72.7%, ctx 77.7%, cro 77.7%, cip 65.6%, ipm 9.5%, tzp 49.4%). *Enterobacter aerogenes* (amc 88.8%, cfp 88.8%, ctx 88.8%, ctx 88.8%, cro 88.8%, cip 88.8%, ipm 37.1%, tzp 55.5%). *Citrobacter freundii* (amc 93.9%, cfp 60%, ctx 66.6%, cro 66.6%, cip 89%, ipm 6.6%, tzp 53.5%). Serratia marcescens (amc 83.3%, cfp 83.3%, ctx 100%, cro 100%, cip 44%, ipm 37.1%, tzp 25%).
Conclusions: For all Enterobacteriaceae imipenemul was the most active beta – lactam, followed by piperacillin/tazobactam. This study showed the trend to increasing resistance of Enterobacteriaceae to beta – lactams and ciprofloxacin

P1527 Analysis of the gastrointestinal system pathogens, 1987 and 2002, according to their number, genus and susceptibility patterns

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Objective and method: In the year 1987, 660 stool specimens and in 2002, 1283 stool specimens had been investigated for the gastrointestinal system pathogens at the Haseki Education and Research Hospital.
Result: The growth rate decreased to percentage 3.3 in 2002 while it was percentage 9.5 in 1987. Most of the growing microorganisms (65%) had been found to be *Shigella* spp. through the year 1987, though the *Salmonella* spp. formed the majority by 74% in 2002. The mostly identified microorganism was *S. paratyphi* B in 1987 while it was *S. typhi* in 2002. *S. typhimurium* rate has increased from 4.5% in 1987 to 18% in 2002. The majority of the *Shigella* spp. had been found to be *S. flexneri* in both years. *S. boydii* strains had formed 31% in 1987 and it decreased to 9% in 2002. While there is no difference between the susceptibility patterns of *S. typhi* in 2 years, there is a clear increase of group *B. salmonella* resistance to ampicillin and ampicillin/sulbactam.
Conclusion: The decreased growth rates through a period of 15 years is pleasing to see the improvement of community hygiene while the increase of bacterial resistance is very scaring since it causes difficulty in treatment.

P1528 Antibiotic susceptibility and the developing resistance within years of *Shigella* strains

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Objectives: Middle socio-economic class of Istanbul forms the patient population of Haseki Education and Research Hospital. The stool samples in 10-year period of time have been analyzed for *Shigella* spp. and resistance problem that develops along years has been put forward within this work.
Methods and result: Five hundred and eight *Shigella* strains isolated from 23.556 stool cultures between the years 1993 and 2002 were identified by conventional methods. When we look at the distribution of the species, *S. flexneri* strains are the most frequent ones with a 61% and *S. sonnei* strains are second with a percentage of 30. Analysing the months during which the *Shigella* strains were isolated, August and September are the most frequent. Analysing the years, a gradual decrease was perceived except 1998. The antibiotic susceptibility of 508 *Shigella* strains with disk diffusion method, using Mueller-Hinton medium according to the recommendations of NCCLS document M2A7 were analyzed. 231 strains (45.4%) were found resistant to ampicillin; 118 strains (23.2%) to ampicillin/sulbactam and 214 strains (42.1%) to chloramphenicol. A higher resistance was seen in *Shigella flexneri* while there was no resistant strain of *Shigella boydii*. The resistance percentages of 56 strains isolated in 1993 for ampicillin, ampicillin/sulbactam and chloramphenicol were 57.1, 17.8 and 0, respectively; while the percentages for 11 strains isolated in 2002 were 81.8, 54 and 63.6, respectively.
Conclusion: The increase of resistance along years is the messenger of possible difficulty in treatment, although the decrease in numbers thanks to the development of hygiene in community. This increase also necessitates the regional follow up of the bacterial resistance.

P1529 Study of incidence and antimicrobial susceptibilities of *Shigella* serotypes isolated from shigellosis cases in an Iranian hospital (2000–2001)

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Background: Shigellosis is the most communicable bacterial diarrheas in our country specially in warm seasons.
Objective: Determining incidence of common *shigella* species and their antibiotic resistance pattern with two standard methods.
Materials and methods: Direct examination and cultivation of diarrheal stool specimens, biotyping culture-confirmed cases with classical biochemical tests and serotyping them for differentiation common *shigella* spp. then performing antimicrobial susceptibility tests by two standard methods:

- broth dilution method for determining minimal inhibitory concentration (MIC) NCCLS approved method.
- agar disk diffusion (NCCLS standard method).

Results: From 89 confirmed cases, *Shigella sonnei* was the serotype most commonly associated with diarrheal disease (40 cases = 44.9%) *Shigella dysenteriae* was the least commonly recoverd species (three cases = 3.3%) More than 95% isolated were sensitive to ciprofloxacin, cefotaxime, and ceftriaxone. The MIC values were (0.25–2) and (4–16), respectively. Seventy percent were sensitive to nalidixic asid (MIC = 8–32) and less than 50% to Tetracyclin, Cotrimoxazol, Ampicilin. The MIC values were (8–16) and (4–32) (16–64), respectively.
Conclusion: Our therpeutic recommendation is to stop prescription of ampicilin, cotrimoxazol, tetracycline, for shigellosis cases. We suggest nalidixic acid for out patients and mild cases, quinolones and third generation of cephems for more acute as a first choice of treatment.

P1530 Seven hundred and twenty-three *Salmonella* strains and their antibiotic resistance pattern

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Objective: Seven hundred and twenty-three Gram-negative bacteria were identified as *Salmonella* spp. with conventional, cultural and serological methods from stool samples sent to the Haseki Education and Research Hospital microbiology laboratory between 1993 and 2002.
Methods: Among the 723 Salmonella isolates most of them were *S. typhimurium* and *S. typhi*, respectively. The two nosocomial outbreaks in 1995 and 1996 in the Pediatric Clinic were responsible for the *S. typhimurium* abundance. For the year 1998, the increase of *S. typhi* isolates is remarkable. The susceptibility of *S. typhimurium* strains to ampicillin, ampicillin/sulbactam and chloramphenicol were determined with Kirby-Bauer disk diffusion technique on Mueller-Hinton medium according the NCCLS document M2A7 recommendations.
Result: The highest resistance against ampicillin, ampicillin/sulbactam and chloramphenicol were detected in *S. typhimurium*. Although among the outbreak strains the resistance pattern for the three antibiotics was 100, 93.3 and 91%, respectively; among the nonoutbreak strains it was 64, 51 and 67%, respectively. No resistance was detected among the *S. paratyphi* A strains.
Conclusion: The increasing drug resistance of these frequent bacterial agents of gastrointestinal system infections is remarkable because of their effect to the public health.

P1531 Antimicrobial resistances in *Campylobacter jejuni/coli* in Italy during 2002: national study of Campyg Working Group of Italian Association of Clinical Microbiologists

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Objectives: Inside a policentric study concerning intestinal infections by *Campylobacter jejuni/coli* we wanted to lay stress on antimicrobial resistances in these pathogens.
Methods: During 2002, eight laboratories sent own strains of *Campylobacter* spp. to central laboratory of Perugia, where they were identified, biotyped and processed for antimicrobial resistances, using modified agar diffusion method on blood agar, as recommended.
Results: We studied 205 strains: 180 *C. jejuni*, 22 *C. coli*, three other ones. We observed these resistances: 51.1% to nalidixic acid; 49.4% to ciprofloxacin, ofloxacin, norfloxacin; 43.3% to tetracycline; 40.0% to minocycline; 2.2% to erythromycin; 1.1% to rokytamicin; 0.6% to gentamycin; no resistances to chloramphenicole. The resistances to new quinolones showed a range from 31.3 to 80.7%.
Conclusion: We confirm the low or very low resistances to macrolides, an increasing resistances to tetracyclines, a still increased resistances to quinolones. We must do a surveillance of this aspect, because invasive enteritis usually by *C. jejuni* need of antimicrobial therapy.

P1532 Bacteriological profile of a fastidious gram-negative organism. Antimicrobial sensitivity pattern of *Serratia marcescens* as assessed by the evaluation of 1004 consecutive cultured strains

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Objective: To investigate the in vitro antimicrobial resistance profile of *Serratia marcescens*, a gram-negative organism of growing importance in the immunocompromised and hospitalized host, and in the extreme life ages.
Methods: All consecutive *S. marcescens* isolates identified in the years 2000 and 2001 were tested against a broad panel of antibiotic compounds, including ampicillin, amoxicillin-clavulanate, cephalothin, gentamycin, amikacin, ciprofloxacin, and cotrimoxazole.
Results: One thousand and four *S. marcescens* strains cultured from clinical specimens of various origin were identified and later tested for antimicrobial sensitivity through standardized laboratory techniques, according to the updated NCCLS recommendations. The cumulative susceptibility pattern showed a complete (100% of tested strains) sensitivity to gentamycin, amikacin, ciprofloxacin, and cotrimoxazole, as opposed to a complete (100%) resistance to ampicillin, amoxicillin-clavulanate, and cephalothin. No significant variations occurred when comparing the year 2000 profile with the year 2001 one.
Conclusions: *Seratia marcescens* deserves careful attention by clinicians, pediatricians, and microbiologists, due to its environmental source, and its tendency to cause nosocomial disease (also as hospital outbreaks), usually interesting immunocompromised hosts, neonates, and the elderly, or patients who underwent surgery and other invasive diagnostic or therapeutic procedures. Third-generation cephalosporins, carbapenems, and fluoroquinolones are the recommended first-choice therapy (The Sanford Guide to Antimicrobial Therapy 2002; Bartlett JG, Pocket Book of Infectious Disease Therapy 2002), followed by anti*Pseudomonas* semisynthetic penicillins, aztreonam, and gentamycin. According to our extensive experience, carried out on more than 1000 *S marcescens* strains, aminoglycosides such as gentamycin or amikacin, fluoroquinolones like ciprofloxacin, and even cotrimoxazole may represent a first-line choice, encompassing also a more favorable pharmacoeconomic profile compared with third-generation cephalosporins and carbapenems, pending in vitro susceptibility testing. On the other hand, an empiric treatment with ampicillin, first-generation cephalosporins, and also amoxicillin-clavulanate is borne by a high risk of clinical and microbiological failure, due to the elevated in vitro resistance profile showed by *S. marcescens* against these last antimicrobial compounds.

P1533 Determination of minimal inhibitory concentration of trimethoprim sulfamethoxazole against *Stenotrophomonas maltophilia*

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Objectives: To determine the in vitro activity of trimethoprim sulfamethoxsazole against *Stenotrophomonas maltophilia* isolates.
Methods: Twenty-nine isolates obtained from blood (17), wound (7), respiratory specimen (5) were tested against trimethoprim sulfamethoxazole. The agar dilution method was performed according to the National Committe for Clinical Laboratory Standards (NCCLS), 2002. *Pseudomonas aeruginosa* ATCC 27853 was used for quality control.
Results: Only one of the 29 isolates is resistant to TMP-SMX. The same strain was resistant by disk diffusion method. MIC_{50} of the isolates is 1 μg/mL, MIC_{90} of the isolates is 2 μg/mL.
Conclusion: *Stenotrophomonas maltophilia*, an opportunistic pathogen, has risen to prominence during the last few years. Its unique antimicrobial susceptibility pattern guides to therapy. Resistance to TMP-SMX varies from 0% to 33.4% in the literature. Since the resistance rate is only 3% according to our results TMP-SMX seems to be appropriate for the infections with *S. maltophilia*. The disk diffusion results for TMP-SMX usually correlate well with the MIC studies. In our study, there was only one resistant strain which was also noticed resistant by disk diffusion.

P1534 Biotypes and antimicrobial susceptibilies of *Brucella* isolates

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Brucellosis remains a public health problem especially in the Mediterranean countries. It is a common infectious disease in rural area of Turkey. In this study, 41 *Brucella* strains isolated from blood and cerebrospinal fluid (CSF) cultures of adult patients with brucellosis were identified to species level and biotypes were detected. *Brucella* species were identified on the basis of typical Gram staining (faintly staining and minute coccobacillus), CO_2 requirements for growth, production of H_2S and urease, growth in the preciseness of aniline dyes, susceptibility of Tbilisi bakteriophage and agglutination in antisera. All of the isolates were *Brucella melitensis*: Two strains of *Brucella melitensis* biotype-1 and 39 strains of *Brucella melitensis* biotype-3. In vitro activities of these strains were detected by *E*-test (AB Biodisk) method. According to the MIC_{90} values, the most active agent was found to be doxycycline (MIC_{90} 0.064 μg/mL) followed by ciprofloxacin (MIC_{90} 0.25 μg/mL), thrimethoprim/sulphamethoxazole and ceftriaxone (MIC_{90} 0.38 μg/mL). Rifampin (MIC_{90} 0.75 μg/mL) exhibited the highest MIC_{90} value (Table 1).

Table 1

Antibiotic	Range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
TMP/SMX	0.064–1	0.094	0.38
Ciprofloxacin	0.064–0.38	0.125	0.25
Rifampin	0.047–2	0.50	0.75
Ceftriaxone	0.047–2	0.125	0.38
Doxycycline	0.023–0.25	0.047	0.064

In conclusion, this study suggests that conventional anti*Brucella* antibiotics are active against *Brucella* species in vitro. Thus routine antibiotic susceptibility tests for *Brucellae* are not necessary. However, antimicrobial resistance must be examined periodically.

P1535 Antibiotic resistance of *Acinetobacter baumanii*: a study on 101 clinical isolates

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Objective: To assay the antibiotic resistance of clinical isolates of Acinetobacter baumanii in order to provide evidences for prudent chemotherapy and clues for further studying on the resistance.
Methods: Hundred and one strains of *A. baumanii* were isolated from clinical specimens from January 2002 to December 2002 in West China Hospital (a national Medical Center with 3000 beds) and collected. Antimicrobial susceptibility testing was performed by the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards. Muller-Hinton agar was used for susceptibility testing. Fourteen antimicrobials ansd sulbactam were included in this study.
Results: Resistant proportions of the 101 clinical isolates against 14 antimicrobials are below (also see Table 1): meropenem 4%; piperacillin-tazo-

Table 1 Antimicrobial susceptibility of 101 clinical isolates of *Acinetobacter baumanii*

	MIC_{50}	MIC_{90}	R	I	S
Ampicillin	512	>512	71	10	20
Piperacillin	128	512	46	22	33
Piperacillin-tazatobactam	64	128	4	57	40
Subactam	8	32			
Cefotaxime	128	512	55	19	27
Ceflazidine	32	256	47	5	49
Cefepime	16	32	11	8	82
Cefoxitin	>512	>512	68	13	20
Imipenem	0.125	2	5	1	95
Meropenem	0.5	2	4	3	94
Aztreonam	16	512	16	35	50
Gentamicin	>512	>512	71	0	30
Amikacin	>512	>512	56	5	40
Ciprofloxacin	32	128	53	2	46
Trimethoprim-sulamethoxazole	304/16	>608/32	74	0	37

bactam 4%; imipenem 5%; cefepime 11%; aztreonam 16%; piperacillin 46%; ceftazidime 47%; ciprofloxacin 53%; cefotaxime 55%; amikacin 56%; cefoxitin 68%; ampicillin 71%; gentamicin 71%; and sulfamethoxazole-trimethoprim 74%. The data of MIC_{90}, MIC_{50}, intermediate and susceptibility is available in Table 1.

Conclusions: Clinical isolates of *A. baumanii* from Western China are mostly resistance to co-SMX, gentamicin and ampicillin, but less to meropenem and imipenem. However, the resistance to carbapenems is found in several isolates. The study on the mechanism of the resistance to carbapenems is following.

Mechanisms of resistance 2

P1536 Selection and characterization of fluoroquinolone resistant *Pseudomonas aeruginosa* by ciprofloxacin (Cpx) and levofloxacin (Lfx) using high-density cultures

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Background: The mutant prevention concentration (MPC) has been proposed as a novel parameter designed to differentiate between compounds propensity to select for and inhibit resistant mutants present in large heterogenous susceptible cultures. When MPC results are applied to FQ pharmacokinetics duration of the dosing interval above the MPC can be calculated (T > MPC). We examined by mutant selection curves (MSC) the different allelic variants of PA mutants selected by Cpx and Lfx and determined whether resistant phenotypes predominate throughout large heterogenous susceptible populations or if their appearance correlates within a defined concentration spectrum.
Methods: Two susceptible strains of PA were chosen (MIC = Cpx 0.125, Lfx 0.25 mg/L). One strain was a genetically constructed mexA-efflux negative lab strain. The susceptible strain was inoculated into Mueller-Hinton broth and grown overnight to produce high density culture ($>10 \times 10$ cfu/mL). Aliquots of $1.7 \times 10 \times 10$ cfu/mL were applied to agar plates seeded with FQ antibiotic (range 0.01–20 mg/L) and incubated for 24 h. Individual colonies were counted. Selected colonies from various concentration points were selected from *gyrA* and *parC* gene sequence analysis.
Results: No differences in the number of cells recovered from Cpx and Lfx plates were observed at concentrations between 0.01 and 0.125 mg/L. For drug concentrations between 1.5 and 7 mg/L, the number of recovered cells on Lfx plates (log reduction −2.39 to −7.36) was 2.5–22 000 times greater than the number of cells recovered from Cpx plates (log reduction 5.82–7.25). Between concentration of 8–16 mg/L, 3–66 cells were recovered from L plates. No colonies were recovered from Cpx plates above a concentration of 6 mg/L. Testing of inoculum of $1.56 \times 10 \times 5$ cfu/mL of the same strain yielded no significant differences in the number of colonies recovered at various concentrations. Sequence analysis of QRDR region from colonies recovered from Lfx plates revealed a *gyrA Ile83* mutant. No confirmed QRDR mutants were recovered from colonies present on Cpx plates.
Conclusion: Selection of resistant PA was greater for Lfx than Cpx when 10×10 cfu/mL culture was sampled. No difference in the selection of mutants were observed with a 10×5 cfu/mL sample of the same culture. QRDR mutants are selected at high concentrations of Lfx when 10×10 cfu/mL sampled. Based on data analyzed to date no QRDR mutants were selected by any concentration of Cpx.

P1537 Rapid selection of *Pseudomonas aeruginosa* with decreased ciprofloxacin susceptibility by serial exposure to new antigram-positive quinolones

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Background: Ciprofloxacin (CIP) is more active against Gram-negative than Gram-positive bacteria. In Gram-positive bacteria, CIP rapidly selects for resistant derivatives that are cross-resistant to newer quinolones with an improved antiGram-positive spectrum. Conversely, newer antiGram-positive quinolones are less active against Gram-negative bacteria. Thus, the question arises as to whether the new antiGram-positive quinolones might favor the selection of resistant Gram-negative organisms which become cross-resistant to CIP.
Methods: Five isolates of *P. aeruginosa* were used. They were either plated (109 CFU/plate) on drug-containing agar to determine the minimal drug concentration inhibiting the emergence of resistant mutants (mutant prevention concentration or MPC), or serially exposed to increasing drug concentrations in liquid cultures. Drugs included CIP, levofloxacin (LVX), gemifloxacin (GFX), moxifloxacin (MFX), or garenoxacin (GRN). The MPC and the increase in MIC were followed, and cross resistance between anti-Gram-positive quinolones and CIP was determined.
Results: The MPC for 90% of *P. aeruginosa* isolates (MPC90) was 1 mg/L for CIP, 8 mg/L for LVX, 16 mg/L for GFX, and 32 mg/L for both MFX and GRN. After serial exposure, all quinolones selected for resistance. However, selection was faster with newer MFX, GFX, and GRN. Resistant *P. aeruginosa* selected by these drugs had a 2–4× increase in their MIC of CIP.
Conclusion: The frequency of resistant *P. aeruginosa* was much higher on antiGram-positive quinolones than on CIP. Moreover, derivatives resistant to new antiGram-positive quinolones had 2–4 times increases in their MIC of CIP. Serial passage in liquid cultures confirmed these results. Blood and tissue concentrations preventing resistance (i.e. >8 mg/L) are not reached in vivo with recommended dosages of newer anti-Gram-positive quinolones. This suggests that treatment with newer quinolones might promote populations of CIP-resistant colonizing pseudomonas in the patient.

P1538 Characteristics of resistance phenotypes of *Pseudomonas aeruginosa* hospital strains

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Background: Recently there have been more frequently isolated *Pseudomonas aeruginosa* strains resistant to many antibiotics, comprising carbapenems. Many different mechanisms are connected with resistance of these bacteria. The production of metallo-beta-lactamases is responsible for the carbapenem-resistance of Gram-negative bacterial species such as *P. aeruginosa* and *Serratia marcescens*. The aim of our study was the analysis of the occurrence and sensitivity to the different kind of antibiotics *P. aeruginosa* strains isolated from patients treated in Rydygier's Hospital in Kraków. Then the detection of metallo-beta-lactamases among carbapenem-resistant strains was carried out.
Material: The study population was the patients hospitalized from 1998 to 2000 into five wards. From 2482 *P. aeruginosa* strains isolated from blood, urine, wounds and sputum 691 were selected and then 187, were fully tested.
Methods: The strains were identified in the automatic ATB system using ID 32 GN strips (bioMerieux). The sensitivity of *P. aeruginosa* strains to different drugs was tested with disc diffusion method (inerpretation according to NCCLS). The following antibiotics were tested: ticarcillin, piperacillin, piperacillin/tazobactam, ticarcillin/clavulanian acid, cefotaxime, ceftazidime, imipenem, meropenem, amikacin. Two discs containing ceftazidime, imipenem and filtered disc containing EDTA or 2-mercaptopropionic acid (2MPA) for detection metallo-beta-lactamates were used.
Results: *P. aeruginosa* strains isolated in the period of three years showed relatively high resistance to antibiotics from penicillin group (respectively to ticarcillin in the range from 59.9 to 87%, and piperacillin from 63.8 to 81.4%). The growth of resistance to ceftazimide was 59% in 2000 years (9% in 1998). The resistance to imipenem and meropenem was about 22 and 26%, respectively, and has grown in analyzed period too. The resistance to carbapenem was metallo-beta-lactamase depended. We isolated strains of *P. aeruginosa* of resistance phenotype to all investigated drugs, in the range from 21.5 to 18.4% in 1998–2000.
Conclusions: The present data indicates that the carbapenems are the most suitaible antibiotics for treatment of infection caused by *P. aeruginosa*. We also showed mechanism of resistance strains to carbapenems with connection to production of metallo-beta-lactamases. Therefore the methods for detection of this enzyme can be very helpful in daily clinical laboratory testing.

P1539 In vitro susceptibility and population analysis of staphylococci after serial passage at sub-MIC levels of dalbavancin and other glycopeptides

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Objectives: Dalbavancin (DAL) is a novel, semisynthetic glycopeptide, with activity against Gram-positive organisms, including resistant strains. It is more active in vitro and in animal models than vancomycin or teicoplanin. In a phase 2 study dalbavancin was well tolerated; weekly doses were effective in deep skin and soft tissue infections and comparable to standard of care. DAL is currently in phase 3 clinical development as a weekly regimen. Staphylococci can express heterotypic resistance to glycopeptides. An increased resistant subpopulation may be an initial step towards the development of resistant strains. This study was designed to determine whether serial passage in vancomycin (VAN), teicoplanin (TEI), or DAL increases the glycopeptide-resistant subpopulation of *S. aureus* (MSSA) or *S. haemolyticus* (SHA).
Methods: MICs of VAN, TEI and DAL were determined after each of 24 serial passages, using preceding 0.5X MIC tube as source of inoculum. Colonies from the passages were selected to assess their resistance phenotype. MICs were determined by standard methods. The resistant subpopulation was calculated as [log10 cfu/mL on BHIA + 4 mg/L drug]/[log10 cfu/mL on BHIA].
Results: For VAN passage mutants, the MIC increased from 0.5 to 2 mg/L in MSSA and the VAN-resistant subpopulation increased from 10 to 6.10-4.3. In SHA, VAN MICs increased from 0.5 to 1 mg/L and the resistant subpopulation increased from 10 to 6.8-10-3.4. After passage in TEI, mutants in MSSA and SHA had MICs of 8 and 16 mg/L, respectively, and the population was homogeneously resistant. After passage in DAL, the MIC increased from 0.25 to 0.5 mg/L (MSSA) and from 0.125 to 0.5 mg/L (SHA). For both MSSA and SHA the resistant subpopulations remained low (*10–7) after 24 passages in DAL.
Conclusions: Long-term sub-MIC passage in glycopeptide results in higher MICs of VAN, TEI and DAL in both MSSA and SHA, although DAL MICs remained *0.5 mg/L. The resistant subpopulation in both VAN and DAL passaged mutants increased, but the subpopulation was larger after selection in VAN. Passage in TEI readily selected for increased resistance to TEI. Staphylococci have a heterotypic response to DAL but it is not easy to increase the resistant subpopulation by continuous exposure to sub-MIC. Since projected trough levels of DAL are over MIC, in vivo selection for clinically relevant resistance to DAL may be more difficult than for other glycopeptides.

P1540 Determination of the minimal inhibitory concentration (MIC) and mutation prevention concentration (MPC) for garenoxacin against clinical isolates of *Streptococcus pneumoniae*

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Objective: Garenoxacin (GRN) is a novel des-F(6)-quinolone. The MPC is an unique measurement of antimicrobial potency as it defines the drug concentration threshold that would require a microorganism to simultaneously possess two or more mutations for growth in the presence of the drug. Antimicrobial resistance with SP is a global pandemic and is defining an important role for fluoroquinolones (FQ) for therapy against this organism, however, emerging resistance to older agents is a concern. We determined the MIC and MPC for clinical isolates of SP against GRN.
Methods: The MICs were determined by microbroth dilution with interpretation based on NCCLS guidelines. For MPC testing,approximately 10×10 cells were applied to GRN plates containing drug and incubated for 24 and 48 h. The lowest concentration that prevented growth was recorded as the MPC.
Results: To date, 344–384 pneumococcal isolates have been tested by both MIC ($n = 384$) and MPC ($n = 344$) for GRN. The MIC_{50} (mg/L), MIC_{90} (mg/L) and range (mg/L), respectively, were as follows: 0.063, 0.125, <0.008–2 mg/L. The modal MIC was 0.03 mg/L. The MPC50 (mg/L), MPC90 (mg/L) and range, respectively, were as follows: 0.125, 0.5, 0.031–≤1 mg/L. The modal MPC was 0.125 mg/L. No differences in GRN MIC or MPC values were seen between isolates that were sensitive, intermediate or resistant to penicillin. A total of 14 clinical isolates with MPCs to levofloxacin (4 to >64 mg/L) were genetically characterized and four contain both *parC* & *gyrA* mutations while the remainder had mutations in the *parC* gene, some of which have not previously been associated with FQ. MIC values for GRN against these isolates ranged from 0.032 to 0.25 mg/L (MIC_{90} 0.25 mg/L) and MPC values ranged from 0.64 to 1 mg/L (MPC90 0.25 mg/L) for 13/14 isolates (≥1 for 1 isolate). MPC values for four isolates with *parC* and *gyrA* mutants were 0.25, 0.5 and 1($n = 2$) mg/L.
Conclusion: GRN is an investigational drug with enhanced in vitro activity against SP. Low MPC values suggest that this compound is less likely to select for FQ resistance. The GRN MPC values were within clinically achievable concentration for isolates with high MPC values to levofloxacin suggesting GRN may be useful for therapy in patients with levofloxacin resistant mutants. GRN looks like a promising new agent for treating SP infections.

P1541 Genetic mechanisms of macrolide resistance in *S. pneumoniae*

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Objective: to analyze the genetic mechanisms responsible for macrolide resistance in *S. pneumoniae*
Methods: Two hunderd and forty-three erythromycin resistant clinical *Streptococcus pneumoniae* isolates collected between october 1999 and februari 2000 were analyzed. MIC's of all isolates for erythromycin, telithromycin and clindamycin were determined via agar dilution. PCR was used to determine the presence of *ermB* and *mefA*. Erythromycin-susceptible strains and erythromycin-resistant strains with known genotype (strain 02J1095 (ermB) and 02J1175 (mefA)) were included in every run as positive and negative controls. Strains without ermB or *mefA* genes or strains with both genes were retested to confirm the genotype
Results: 84.3% of the isolates had only the erm gene and these strains all displayed an MLSB phenotype; 5.8% had only the *mef* gene and these strains all displayed the M phenotype. 3.3% had erm plus *mef* genes and 6.6% had neither erm nor *mef* genes. These strains had varying genotypic and phenotypic resistance patterns. The ketolide telithromycin retained good activity against most *ery-R* strains; the increase in MIC for telithromycin was most pronounced in *mef*-positive strains.
Conclusion: Compared with an earlier analysis of pneumococci collected in Belgium between 1995 and 1997, there was an increase in *ery-R* strains without mef nor *erm* genes. Significant regional differences were observed between the northern part and the southern part of Belgium. In the northern part, erm containing isolates were significantly less present than in the Southern part (78.2 vs. 90.6%) and *mef* and double negative strains were clearly more present (11 vs. 2% for mef and 8.2 vs. 4.7% for double negative strains).

P1542 Identification of macrolide-resistance mechanisms and distribution of efflux gene variants in Canadian isolates of *Streptococcus pneumoniae*: results of a 5-year (1997–2002) National Surveillance Study

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Objectives: Macrolide resistance in *S. pneumoniae* is largely attributed to either target modification or active drug efflux. Ribosomal methylation mediated by the *erm(B)* gene confers cross-resistance to macrolides, lincosamides and streptogramin B antibiotics (MLSB phenotype), while macrolide efflux (mef) genes confer low-level resistance to only 14- and 15-membered macrolides (M phenotype). Two variants of the *mef* gene, *mef(A)* and *mef(E)*, have recently been shown to reside on different genetic elements, Tn1207.1 and the macrolide efflux genetic assembly (mega) insertion element, respectively. The purpose of this study was to evaluate the incidence of *erm(B)* and mef genotypes among Canadian isolates of macrolide-resistant *S. pneumoniae* and to determine the prevalence of *mef(A)* and *mef(E)* gene variants among these isolates.
Methods: Five hundred and ninety-four macrolide-resistant (erythromycin MIC, >1 μg/mL) *S. pneumoniae* isolates were collected between 1997 and 2002 as part of an ongoing Canadian Respiratory Organism Susceptibility Study (CROSS). Susceptibility testing was performed by the NCCLS-approved broth microdilution method and all isolates were evaluated by PCR for the presence of *erm(B)* and mef genotypes. PCR-RFLP analysis was

performed on 104 randomly selected *mef*-positive isolates to discriminate between *mef(A)* and *mef(E)* gene variants.
Results: Two hundred and ninety-eight out of 594 (50.2%) *S. pneumoniae* isolates were resistant to macrolides alone (M phenotype) and carried the *mef* gene. The *erm(B)* genotype was detected in 259 (43.6%) resistant isolates. 18 (3.0%) isolates were PCR-positive for both *mef* and *erm(B)*, while 19/594 (3.2%) isolates were negative for all mechanisms tested. Of the 104 macrolide-resistant mef-positive *S. pneumoniae* isolates analyzed by PCR-RFLP, 98 (94.2%) carried the mef(E) gene variant. The mef(A) gene was detected in only 6/104 (5.8%) isolates.
Conclusions: *Erm(B)* and *mef* genotypes are equally prevalent among Canadian isolates of macrolide-resistant *S. pneumoniae* and have remained stable throughout the 5-year study period. Although both *mef* gene variants have been identified in clinical *S. pneumoniae* isolates, the mega insertion element is the primary genetic element that carries the macrolide efflux gene in Canadian isolates of macrolide-resistant *S. pneumoniae*.

P1543 Rate and mechanism of resistance to linezolid in *Streptococcus pneumoniae* susceptible or resistant to penicillin G or macrolides

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Background: The oxazolidinone linezolid (LIN) inhibits translocation of the peptide chain from the A site to the P site of the ribosome. LIN resistant mutants, which are not cross-resistant to other drug classes, display mutations in the 23S rRNA at position 2447 (*E. coli* numbering) in *Staphylococcus aureus* and 2576 in *Enterococcus fecium* and *fecalis*.
Objectives: We evaluated the acquisition of resistance to LIN, erythromycin (ERY) and spiramycin (SPI) of *S. pneumoniae* with different levels of resistance to penicillin G (PEN) and studied the mechanism of LIN resistance
Methods: 20 *S pneumoniae*, 5 susceptible (S) to PEN (MIC < 0.064 mg/L) and ERY; 5 PEN S and resistant (R) to ERY; 5 intermediate (I) to PEN (MIC: 0.125–1 mg/L); 5 PEN R (MIC > 1 mg/L) were passaged daily in subinhibitory concentrations of LIN, ERY and spiramycin (SPI), a 16-membered macrolide. The four copies of the 23S rRNA rrl gene of LIN R mutants were amplified separately and their sequence determined.
Results: mean of number of passages necessary to reach resistant level (R) to the selecting agent:

Table 1

	PEN S ($n=10$)			
	ERY S ($n=5$)	ERY R ($n=5$)	PEN I ($n=5$)	PEN R ($n=5$)
ERY	24.4 ± 16.8	–	21.8 ± 14.8	8 (1 strain)
SPI	20.8 ± 8.0	–	$11.4 \pm 3.5^*$	7 (1strain)
LIN	$\geq 58.6 \pm 10.3^*$	$16.6 \pm 7.5^*$	44.4 ± 18.9	$21.8 \pm 10.3^*$

$^*P < 0.05$.

LIN R mutants had G2576U mutation in the four copies of the 23S rRNA.
Conclusion: LIN showed the lowest rate of acquisition of resistance and the mutants did not display cross-resistance to other drug classes (macrolides, chloramphenicol, tetracyclines, cotrimoxazole, fluoroquinolones). Strains resistant to ERY or PEN acquired more rapidly resistance to LIN. Resistance to LIN was secondary to mutation G2576U in domain V of 23S rRNA.

P1544 Resistance mechanisms to macrolide antibiotics in erythromycin-resistant *Streptococcus pneumoniae* in Turkey

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Objective: To determine the macrolide resistance mechanisms of *S. pneumoniae* isolated from different regions of Turkey.
Methods: A total of 151 clinical *S. pneumoniae* (11 highly and, 93 intermediately penicillin-resistant, 47 penicillin-susceptible) isolates, recovered at 7 medical centers located in different cities, were selected randomly for the study. Erythromycin (E) and clindamycin (CC) susceptibilities were investigated by disk diffusion according to the NCCLS recommendations. MIC values were determined by the *E*-test (AB Biodisk). The *erm (B)* and *mef (A)* genes were detected by PCR using specific primers. Clonal relatedness of the isolates were analyzed by BOX-PCR using BOX-A1 primer.
Results: Overall, 40 (26.4%) isolates were resistant to erythromycin. Of these, 37 (92.5%) were highly ER with MIC values of >256 mg/L. The most common mechanism (38/40) was target site modification due to *erm (B)* genes. Only two isolates harbored mef (A) efflux gene. All but one erm (B) positive isolate, were also resistant to CC, suggesting constitutive MLSB phenotype. The remaining isolate showed inducible resistance phenotype. Macrolide resistance was more common among penicillin nonsusceptible isolates. ER isolates belonged to different clonal groups as determined by BOX-PCR.
Conclusion: The most common mechanism of macrolide resistance in Turkish *S. pneumoniae* isolates, is target site modification due to the presence of erm (B). M phenotype is relatively uncommon.

P1545 A new generation of fluoroquinolone resistant clinical isolates of *Streptococcus pneumoniae*: Can we meet the challenge?

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Objectives: Fluoroquinolone (FQ) resistance in *Streptococcus pneumoniae* is increasing and consequently the prevalence of isolates with a mutation both in the parC and gyrA genes has risen. Although the presence of clinical isolates with more than one mutation in either parC or gyrA has not been reported, studies in vitro have demonstrated that ciprofloxacin (cipro) or levofloxacin (levo) can select for a third-step parC mutation resulting in a greater degree of nonsusceptibility to many FQs. This study was undertaken to determine if the increased levo use since 2000 in Canada, for the treatment of pneumococcal infections, has resulted in the selection of such mutants.
Methods: Since January 2000, 37 strains collected from across Canada showing a cipro MIC $>$ or $= 32$ mg/mL were identified. The quinolone resistance determining regions of the parC and gyrA genes from these isolates were amplified by PCR and the nucleotide sequence determined.
Results: Two isolates with a cipro MIC $>$ or $= 64$ mg/mL and a levo MIC of 16 mg/mL were identified; each with a double parC mutation and a single gyrA mutation. Both were serotype 14 strains and had Ser-79-Phe and Asp-83-Tyr substitutions in ParC. One isolate showed a Ser-81-Phe substitution in GyrA and the other a Ser-81-Tyr substitution. Both strains were isolated from elderly patients (>65 years) with chronic lymphocytic leukemia who had been admitted to hospital suffering from pneumonia and who had been treated unsuccessfully with levo prior to isolation of the strain.
Conclusion: Our finding demonstrates the existence of a new generation of mutants which has emerged in parallel with the increase in FQ usage. However, it is difficult to predict whether or not this trend will continue as new, more potent quinolones are used for treatment.

P1546 Selection of resistance to and mutagenicity of different fluoroquinolones in *Staphylococcus aureus* and *Streptococcus pneumoniae*

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Objective: To analize the capability of levofloxacin, trovafloxacin, gemifloxacin, clinafloxacin, moxifloxacin and ciprofloxacin to select for an increase in the MICs for *S. pneumoniae* and *S. aureus*, as well as to determine the mutagenic activity of the above mentioned fluoroquinolones.
Methods: Two strains of *S. aureus* and five strains of *S. pneumoniae* were used. MICs were determined following NCCLS criteria. The selection of resistance was performed by serial passages with antibiotic concentrations from 3 doubling dilutions below to 3 doubling dilutions above the MIC. For each subsequently daily passage an aliquot containing approximately 5×10^5 bacteria were cultured in the concentrations above mentioned. An aliquot of bacteria that grow at greater concentration was taken and subcultured in a blood agar without antibiotic pressure. Then, approximately 5×10^5 bacteria were cultured again into the next passage containing each diluted drug. Daily subculturing was done until mutants with MICs $>$ 4x to the selecting drug appeared. In each mutant the MIC were done and mutations in the QRDR of the gyrA and parC genes were determined by RFLP (Ser 84 of GyrA and Ser80 of GrlA of *S. aureus*, and Ser81 of GyrA and Ser79 of ParC of

S. penumoniae). The mutagenicity of each fluoroquinolone was performed by the *Salmonella* and *E. coli* assays.
Results: Subculturing *S. aureus* in ciprofloxacin led to the increase in MIC four-fold after 5–7 passages, 14–16 for moxifloxacin, 6–8 for trovafloxacin, 8–11 for clinafloxacin, 12–15 for levofloxacin and 8 for gemifloxacin. To increase four-fold the MIC of ciprofloxacin for *S. pneumoniae* 7–10 passages were needed, 8–12 for moxifloxacin, 7–12 for trovafloxacin, 10–13 for levofloxacin, 8–14 for clinafloxacin and 8–10 for gemifloxacin. Levofloxacin and moxifloxacin were the two fluoroquinolones with lower mutagenic activity.
Conclusion: Our results suggest that at concentrations close to the MIC there is a direct relationship between the selection of resistance and the mutagenic potency of fluoroquinolones, with levofloxacin and moxifloxacin being the fluoroquinolones showing the less selection of mutants and mutagenicity.

P1547 Molecular characterization of fluoroquinolone-resistant *Streptococcus pneumoniae* isolates from Canada

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Objective: Fluoroquinolones are increasingly used in the empiric treatment of respiratory tract infections caused by *S. pneumoniae*. Although the prevalence of fluoroquinolone resistance in *S. pneumoniae* remains low (< 2%), it is essential to continue monitoring resistance rates. The purpose of this study was to characterize the fluoroquinolone-resistant (ciprofloxacin MICs ≥ 4 μg/mL) clinical isolates of *S. pneumoniae* obtained from across Canada throughout 1997–2002.
Methods: Over a five-year period (1997–2002), 75 ciprofloxacin-resistant *S. pneumoniae* isolates were collected from 9 of 10 Canadian provinces as a component of an ongoing annual national respiratory organism surveillance study. Their susceptibility to ciprofloxacin, gatifloxacin, levofloxacin, and moxifloxacin was determined. The quinolone resistance determining regions (QRDRs) of GyrA and ParC were sequenced, and active efflux was evaluated via reserpine studies. The isolates were also serotyped and molecularly fingerprinted.
Results: QRDR mutations in ParC and GyrA were detected in 65/75 (87%) and 38/75 (51%) of the isolates, respectively. The most common mutations were Ser81Phe and Gly85Lys in GyrA and Ser79Phe/Tyr in Par*C*. 25/75 (33%) of the isolates displayed reserpine-sensitive efflux (four-fold or greater reduction in MIC in the presence of reserpine) of ciprofloxacin. Pulsed field gel electrophoresis revealed considerable heterogeneity amongst the isolates.
Conclusions: 87% and 51% of the 75 ciprofloxacin-resistant *S. pneumoniae* isolates had QRDR mutations in ParC and GyrA, respectively. 33% of the isolates were ciprofloxacin-efflux positive. The majority of ciprofloxacin-resistant *S. pneumoniae* isolates were genetically unrelated indicating that the spread of ciprofloxacin resistance is currently not attributable to clonal dissemination in Canada.

P1548 Initial description of the L22 mutation responsible for Quinupristin/Dalfopristin resistance in *Streptococcus pneumoniae*: case reports from the SENTRY Antimicrobial Surveillance Program

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Objective: Although resistance (R) to macrolides and clindamycin remains common and escalating, quinupristin/dalfopristin (Synercid)-R among *S. pneumoniae* strains is extremely rare. Recently Malbruny et al. described an L22 ribosomal protein alteration responsible for Synercid-R in *Staphylococcus aureus*. A similar mutational insertion in L22 was observed in pneumococcal isolates from the SENTRY Antimicrobial Surveillance Program (2001–2002).
Methods: Among *S. pneumoniae* isolates from community-acquired respiratory tract infections in the SENTRY Program (North America, 2001), 158 erythromycin (ER) -R strains were initially screened for erm(B) and mef(A) by multiplex rapid cycle PCR/microwell-format probe hybridization methods. Nine ER-R pneumococci remained negative (USA 6 strains; Canada 3 strains) after PCR screen and were investigated for 23S, L4 and L22 mutations by gene sequencing. An isolate from pleural fluid in a 36-year-old-female living in New York state (17–3167B) had the following MIC (ug/mL) pattern: Synercid 4, quinupristin 8, dalfopristin 128, ER 1, azithromycin 0.5, clarithromycin 1, rokitamycin 2, roxithromycin 16, and clindamycin ≤0.12. A second strain (107–6120) with an identical MLSB resistance pattern was observed in a patient from Kentucky (March, 2002).
Results: A 5 amino acid (AA) insert at the C terminus of the L22 riboprotein was found in both strains. The insert was a duplication of AA 104–107 of L22 (RTAHI) into AA position 108–112. This insert overlaps with the insert (also a duplication) described as the mechanism of Synercid-R previously noted in *S. aureus*. An A2059G mutation in 1 of 4 alleles of the 23S rRNA gene was also detected in strain 17–3167B.
Conclusions: This is the first report of Synercid-R *S. pneumoniae* strains produced by a 5 AA insertion in L22, a mechanism only recently described in *S. aureus* (AAC 46 : 2200–7, 2002). Since all nonerm(B), nonmef(A) MLSB-R isolates occurred in the North American sample, R surveillance in this region should routinely include expanded molecular characterization.

P1549 Differences in selection of efflux-mediated fluoroquinolone resistance in penicillin-susceptible and -resistant *Streptococcus pneumoniae* after repeated exposure to ciprofloxacin and moxifloxacin

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Objectives: Increasing interest in the antipneumococcal activity of fluoroquinolones (FQs) has raised awareness that FQs may differ in their propensity to promote the development and spread of resistance. Efflux, a mechanism that can contribute to reduced FQ susceptibility in *S. pneumoniae* (SP), may represent an important first step in the development of FQ resistance. The current study aimed to investigate the potential of ciprofloxacin (CIP) and moxifloxacin (MXF) to select efflux-mediated resistance.
Methods: Mutants were generated by a selectional step procedure in which 4 strains of SP (2 PenS, 2 PenR) were exposed to inhibitory concentrations of CIP and MXF. Agar dilution MICs were determined for ethidium bromide (EB) and norfloxacin (NOR), two compounds typically used as substrates to monitor efflux-mediated resistance, and the FQs, MXF, CIP, and levofloxacin. Sequencing was used to detect mutations within PCR amplicons representing the promoter and structural gene of pmrA and the quinolone resistance determining regions (QRDRs) of gyrA, gyrB, parC, and parE.
Results: Sequence analysis in 1st step CIP-selected mutants revealed AA changes in ParC (Ser79) in 6 of 7 PenS mutants, whereas 0 of 8 PenR mutants had QRDR mutations. In 1st step MXF-selected mutants, there were no differences in the prevalence of QRDR mutations in GyrA (Ser81) between mutants from PenS (4 of 8) or PenR (3 of 6) parents. After 3 exposures to CIP, 18 of 21 mutants (86%) from all 4 strains exhibited 4- to 16-fold increases in the MIC of EB. In contrast, after 3 exposures to MXF, none of 24 mutants had an increased MIC of EB. Some 3rd step MXF-selected mutants (58%) were more susceptible than the parent strains to EB (MIC decreased from 16 to 2 μg/mL), suggesting that MXF may select reduced efflux. CIP-selected 3rd step mutants had four- to eight-fold higher MICs of the efflux substrate NOR than those selected with MXF. There were no promoter or structural gene alterations in pmrA in any mutants. However, a PenR parent was mutated within the putative −35 and −10 elements of pmrA, and also harbored six different AAs in the structural gene when compared with WT PenS parents.
Conclusions: These data suggest that CIP selected mutants with enhanced efflux while MXF either did not select enhanced efflux or reversed the enhanced efflux phenotype. In addition, PenR parents had a higher propensity for producing an efflux phenotype, which may reflect the differences observed in the pmrA sequence.

P1550 Different contributions of DNA gyrase and topoisomerase IV mutations and pump efflux to fluoroquinolone resistance in clinical isolates of *Streptococcus pneumoniae*

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Objectives: *S. pneumoniae* resistance to fluoroquinolones is acquired in stepwise fashion with the introduction of chromosomal mutations that alter the target proteins, DNA gyrase and topoisomerase IV, or decrease intracellular

drug accumulation by active efflux. This study aimed at assessing the different contributions of such mutations to fluoroquinolone resistance in clinical pneumococci recently isolated in Italy.
Methods: 15 ciprofloxacin-resistant (MIC > 2 mg/L) cultures were identified among 475 clinical isolates of *S. pneumoniae* collected from eight hospitals in central Italy in the course of a 3-year multicenter study (SEMPRE Project). Using suitable methodologies, test strains were characterized for serotype, PFGE, susceptibility to several antibiotics, point mutations in the QRDRs of *gyrA* and *parC*, and the presence of efflux pumps acting through proton- or ATP-driven membrane transporters. Reference strains ATCC 49619 and R6 were used as controls.
Results: All 15 isolates (12 belonging to 10 serotypes and 3 untypable) exhibited different PFGE profiles. Only 1 isolate had intermediate or high-level resistance to all the other fluoroquinolones tested (ofloxacin, ulifloxacin, levofloxacin, sparfloxacin, trovafloxacin, and moxifloxacin); with respect to the other drugs, 12 strains were resistant to 3 or more classes of antimicrobial agents. Mutations in QRDRs were very heterogeneous. Amino acid changes (consistently to F) at both sites GyrA/S81 and ParC/S79 were detected in four double mutants. Another double mutant (the most highly quinolone-resistant isolate) exhibited a K at GyrA/E85 and an F at ParC/S79. Single mutations (consistently to F) were observed both at GyrA/S81 (1 isolate) and ParC/S79 (6 isolates, all intermediate to levofloxacin). Ostensibly, there were no amino acid changes in the remaining three isolates. A multidrug transporter susceptible to reserpine was expressed by nine isolates, including the only one susceptible to CCCP which thus bore both efflux systems. No active efflux was exhibited by the remaining six isolates.
Conclusion: The emergence of fluoroquinolone-resistant clinical *S. pneumoniae* isolates, though still low in incidence, is a cause for concern. Continued surveillance and molecular analysis of resistant pneumococci are essential to predict cross-resistances within this class of antibiotics. However, exposure to ever-novel fluoroquinolones will likely increase the heterogeneity of mutations, further complicating the issue.

P1551 Macrolide resistance mechanisms in *Streptococcus pneumoniae* collected from patients with community-acquired respiratory tract infections in France, Spain and Italy over a 10-year period: The Alexander Project 1992–2001

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Objectives: Phenotypic data from *S. pneumoniae* isolated in 3 European countries participating in the Alexander Project, with high levels of macrolide resistance, showed that the MLS(B) phenotype predominated in the later years of the study but the M phenotype was present in approximately 13.0% of early isolates, suggesting that efflux may once have played a more prominent role. The aim of this study was to determine the distribution of macrolide resistance mechanisms in this population over a 10-year period.
Methods: Macrolide resistance mechanisms were determined by PCR in 1591 isolates of macrolide-resistant *S. pneumoniae*. Isolates were collected over a 10-year period (1992–2001) in France, Spain and Italy from patients with community-acquired respiratory tract infections, as part of the Alexander Project.
Results: Overall, 89.2% of isolates were erm(B), 2.3% mef(A), 0.4% both erm(B) and mef(A), 0.1% erm(A) subclass erm(TR) and 8.0% negative for the mechanisms tested. The genotype distribution was relatively constant over the 10 years in each of the three countries. Erm(B)-positive isolates with erythromycin A MICs ≤16 mg/L were tested for inducible expression of the *erm(B)* gene by the double disc method and were found to have heterogeneous inducible expression of *erm(B)*. This form of inducible expression was prevalent in the early years of the study in France and Spain, but not Italy, and became rare in the later years of the study.
Conclusions: Genotypic analysis showed that efflux was not the cause of the observed M phenotypes early in the study and that these isolates were, in fact, *erm(B)* with heterogeneous inducible expression. These data suggest that the evolution of macrolide resistance in these countries included a stage in which the inducible expression of *erm(B)* was more tightly regulated than at present, and that resistance mechanisms have evolved over time towards greater prevalence of poorly regulated inducible resistance responsible for increased MICs. A total of 8.0% of isolates with the MLS(B) phenotype were shown to be negative for the methylase and efflux genes tested. Further work is needed to determine the mechanisms involved. These are most likely to be ribosomal mutations.

P1552 Comparative minimal inhibitory (MI) and mutant prevention (MP) concentration (C) of gatifloxacin, garenoxacin, levofloxacin and moxifloxacin against 307 clinical isolates of *Streptococcus pneumoniae* (SP)

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Objective: MIC testing determines antimicrobial susceptibility and compares in vitro potency between drugs. MPC is a novel concept that determines the propensity of an antimicrobial compound to select for resistance. It is defined as the antimicrobial drug concentration threshold that would require an organism to simultaneously possess two mutations to grow in the presence of the drug. For community-acquired respiratory tract infections, SP remains a major pathogen and fluoroquinolones (FQ) have become increasingly important for therapy due to increasing pneumococcal resistance to various compounds. Concerns over FQ resistant SP have emerged. We determined MICs and MPCs of 3 FQs (gatifloxacin [GA], levofloxacin [Lfx], moxifloxacin [Mfx]) and garenoxacin (GRN) (an investigational des-F(6)-quinolone [DQ]) against clinical isolates of SP to determine if differences existed between the compounds for their likelihood to select for FQ/DQ resistant SP.
Methods: MIC testing was performed by microbroth dilution in accordance with NCCLS guidelines. For MPC testing approximately 10×10 bacterial cells were applied to agar plates containing drug. Cultures were incubated at 35°C in 5% CO_2 for 24 and 48 h. Drug concentration that prevented growth was recorded as the MPC.
Results: To date 307 clinical isolates were tested. MIC and MPC values were not different against penicillin susceptible or resistant strains so data was combined. The following represents MIC_{50}/MIC_{90} values for GA, GRN, Lfx, Mfx, respectively: 0.25/0.5; 0.063/0.125; 1/1; 0.125/0.25. The MPC50/MPC90 values, respectively, were as follows: 1/2; 0.125/0.5; 2/4; 0.5/1. Time (h) serum drug concentrations are expected to remain above the MIC_{90}/MPC90 (T > MPC90) based on conventional dosing is as follows, respectively: 22/6; >24/>24; 18/3; >24/>24.
Conclusion: Maintaining FQ activity against SP is essential as resistance rates to other compounds increase. Newer FQs (GA, Mfx)/DQ are more potent in vitro than Lfx. By MPC testing, Lfx appears more likely to select for first-step resistant mutants as T > MPC90 was the shortest and MPC values were higher. Both GRN and Mfx maintain T > MPC90 for the entire dosing interval and appear less likely to select for resistance. Based on the MPC model GRN = Mfx > GA > Lfx based on measurements completed to date. Use of the newer FQ/DQ agents with lower MPC values and/or prolonged T > MPC for SP may prolong the SP susceptibility to all FQ/DQ agents.

P1553 Quinolone-resistance mutations in the *gyrA* gene of *Acinetobacter baumannii*

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Objective: The aim of this study was to determine gyrA gene mutations in quinolone- resistant isolates of *A. baumannii* by using PCR-RFLP.
Methods: A total of 36 clinical isolates of *A. baumannii* were incluyed in this study. All isolates were recovered from patients admitted in the Hospital de La Princesa from 1999 to 2001. Isolates were identified as *A. baumannii* using MicroScan System (Dade-Behring). Susceptibility testing to ofloxacin was performed by an agar dilution method according to NCCLS 1999 breakpoints. The mutations in the *gyrA* gene were determined by using PCR amplification of the Quinolone Resistance Determining Region (QRDR). The presence or absence of *gyrA* mutations was determined by the digestion of the PCR products with HinfI. HinfI digestion of the PCR product from a quinolone susceptible isolates generated two fragments of 291 bp and 52 bp, the HinfI restriction site in isolates carrying a mutation at codon 83 was abolished, resulting in no digestion of the fragment containing the full-length PCR product. *A. baumannii* ATCC 19606 was used as control strain for Susceptibility tests and PCR-RFLP study.
Results: Thirty-five isolates were quinolone resistant (CMI range to ofloxacin was16–128 mg/L) and only one strain was susceptible (CMI = 1 mg/L). The HinfI digestion generated one fragment of DNA in all quinolone-resistant isolates and two fragments in the susceptible isolate and in the *A. baumannii* ATCC 19606 strain.
Conclusions: This study showed high level of quinolone-resistance in clinical isolates of *A. baumannii*. Mutations affecting the QRDR of the *gyrA* gene seem to be related with high MICs to quinolones.

P1554 Mechanisms of resistance to quinolones and beta-lactam antibiotics, epidemiology and clinical impact of *Acinetobacter* genomospecies 3 isolated in Spain

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Objectives: The aim of this study was to characterize, in depth, 15 strains of genomic species 3 isolated from 20 hospitals in Spain in November, 2000.
Methods: The strains were identified by amplified ribosomal DNA restriction analysis (ARDRA). In this study, the epidemiological relationship, the antimicrobial susceptibility and the mechanisms of resistance to different antimicrobial agents, such as quinolones and beta-lactams, of these species of *Acinetobacter*, were performed. To achieve this aim, REP-PCR, PFGE, PCR amplification of different groups of beta-lactamases, IEF, and detection of mutations in the QRDR of *gyrA* and *parC* genes were carried out.
Results: The results showed that all the strains were not epidemiologically related and that 60% were able to cause infections. Interestingly, and in agreement with other reports, this species of *Acinetobacter* presented a high susceptibility to most of the antibiotics, contrary to what is described for *A. baumannii*. Sixty-six percent of the isolates were resistant to ampicillin and rifampicin, 20% to azithromycin, 6.6% to piperacillin, cefepime, ciprofloxacin, gemifloxacin and gentamicin and 0% to ceftazidime, sulbactam, ampicillin/sulbactam, imipenem, meropenem, amikacin, tobramycin, tetracycline, doxicycline, minocycline, cotrimoxazol and colistin. Neither amplification nor isolation of any beta-lactamase was possible although, the results of the IEF assay showed a production of a beta-lactamase at $pI > 8$ (probably chromosomal beta-lactamase), thereby suggesting a hyperexpression of the AmpC or a permeability problem in the resistant strains. Regarding quinolones resistance, only one strain was resistant to ciprofloxacin and presented a change from Ser to Leu in the positions 83 of GyrA and 80 of ParC.
Conclusions: *Acinetobacter* genomospecies 3 isolated in Spain are very heterogeneous and highly susceptible to all the antimicrobial agents studied. However, in these isolates resistant to beta-lactam antibiotics and quinolones, the resistance is associated with overexpression of AmpC and with mutations in the *gyrA* and *parC* genes, respectively. Finally, a high percentage of isolates are found to cause infectious diseases.

P1555 Bactericidal activity of fluoroquinolones against *Klebsiella pneumoniae* containing the plasmid-mediated resistance determinant qnr

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Objectives: To evaluate the in vitro bactericidal activity of ciprofloxacin (CIP), levofloxacin (LEV) and moxifloxacin (MXF) against *K. pneumoniae* IMP17 (wild-type strain) and *K. pneumoniae* IMP22 (transconjugant from *K. pneumoniae* IMP17, containing plasmid pMG252 which codes for the plasmid-mediated quinolone-resistance determinant qnr).
Methods: MICs and MBCs of FQ were evaluated by microdilution, according to NCCLS guidelines. Time-killing kinetics were conducted at drug concentration of 4× MIC of the three FQ, with a starting inoculum of 106 cfu/mL, and bacterial counts at 2, 4, 8 and 24 h.
Results: MICs of CIP, LEV and MXF against *K. pneumoniae* IMP17 were 0.5, 0.5 and 0.25 mg/L, respectively. MICs against *K. pneumoniae* IMP22 (containing qnr) were 8 (CIP and LEV) to 32 (MXF) times higher than against *K. pneumoniae* IMP17. MBCs of the three FQ were the same or one dilution higher (CIP against *K. pneumoniae* IMP17) than the corresponding MICs. In the time-killing assay, CIP, LEV and MXF caused at 4 h a reduction in viable *K. pneumoniae* IMP17 (expressed as log10 of cfu/mL) of 3.4, 3.3 and 3.6, respectively. CIP maintained this activity up to 24 h (3.0 log10 reduction), but bacterial regrowth was noted after 8 h for LEV and MXF. Against *K. pneumoniae* IMP22 the reduction in viable organisms caused by CIP, LEV and MXF were 1.4., 1.8 and 1.7 at 4 h, 2.2, 2.3 and 2.3 at 8 h and 3.1, −0,4 and 3.4 at 24 h.
Conclusions: The qnr increases the MICs of CIP, LEV and MXF 8–32 times, but the MBC/MIC ratios of the three agents were not affected. In the time-killing assay the bactericidal activity of CIP and MXF against *K. pneumoniae* expressing qnr was markedly delayed, while LEV did not express bactericidal activity.

P1556 Correlation between the macrolide-resistance phenotypes and genotypes in methicillin-resistant *Staphylococcus aureus* isolates from two Greek university hospitals

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Objectives: To analyze the distribution of the macrolide-resistance genes in 63 methicillin- and erythromycin-resistant *Staphylococcus aureus* clinical isolates collected in two University Hospitals of central and south-western part of the country.
Methods: Methicillin-resistant *S. aureus* isolates (MRSA) were classified into clones by Southern-blot hybridization of ClaI digests with the mecA and Tn554 DNA probes and Pulsed-Field-Gel Electrophoresis (PFGE) of SmaI digests of chromosomal DNAs (ClaI-mecA::ClaI-Tn554::PFGE). The Etest, and the modified disk diffusion method for the characterization of a constitutive or an inducible mechanism determined the resistance phenotypes to erythromycin, clindamycin and lincomycin. The relative frequency of *ermA*, *ermB*, *ermC*, *msrA* and *msrB* genes was investigated by PCR with specific primers. Hybridizations of ClaI DNA digests with the *ermA* and *ermC* genes verified the PCR results and revealed specific patterns.
Results: Resistance to erythromycin was detected in 60.5% of the MRSA clinical isolates. A total of 63 isolates fall into this group 21 MRSA from Larissa and 42 MRSA from Patras. Inducible resistance to erythromycin was detected in 5 isolates, belonging to PFGE types C and F. PCR revealed the presence of *ermC* gene in 58 isolates, *ermA* gene in 4 and both *ermA* and *ermC* in one isolate. No other gene was present from the tested group. Hybridizations of DNA ClaI digests with the *ermC* probe showed the presence of one hybridization band of 2.5 Kb in 21 strains of X′::KK::B, 9 strains of III′::KK::B and 2 strains of II::NH::C clones; one hybridization band of 3.5 Kb in 21 strains of X′::KK::B and 2 strains of III′::KK::B clones; one hybridization band of 12.2 Kb in 3 strains of clone VII′::NH::F. Hybridization with the ermA probe showed the presence 2 bands (3.8/9 Kb) in the strain II::m::B; four bands (3/3,8/5/8.5 Kb) in the strain III::B::E; two bands (3.8/4.1 Kb) in the strain II′::A::A; one band (4.5 Kb) in the strain I::KK::C. The isolate of clone XII::B::B showed one band of 2.5 Kb with the *ermC* gene and two bands (3.8/8.5 Kb) with the *ermA* gene.
Conclusions: Inducible resistance to macrolides in the minority of MRSA isolates correlated with two PFGE types, independently of the presence of ermA or ermC genes. Most of the isolates possessed the *ermC* gene in different DNA bands. The ClaI-ermA hybridization bands correlated with the ClaI-Tn554 hybridization patterns, verifying the presence of the gene on the Tn554 transposon.

P1557 Comparative analysis of 11 genes from isolates of vancomycin-intermediate *Staphylococcus aureus* (VISA) and hVISA

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Background: Comparative genomic analysis of Mu50, a Vancomycin Intermediate *Staphylococcus aureus* (VISA), against N315 (vancomycin sensitive (VSSA), revealed differences in several genes. Six genes (*mutS*, *opuD*, *atl*, *metB*, *prfC* and *modA*) were studied in Mu50 and Mu3 and five genes (*murA*, *SA2486*, *yibM*, *rnr* and *odhA*) were studied in clinical VISA (9) and hVISA (10) and VSSA (11) isolates to identify their role in vancomycin resistance.
Methods: MICs and population analysis were used to confirm VISA/hVISA status. DNA was extracted and used to perform PCR. The resultant amplicons were sequenced and the gene sequences compared with the published sequences in Mu50 and N315 plus the vancomycin sensitive MW2 strain using Clustalw
Results: The sequences in Mu50 and Mu3 of the genes *mutS*, *opuD*, *atl*, *metB*, *prfC* and *modA* were identical to N315. No clinical strain sequences for *yibM* including Mu50 and Mu3 had the published Mu50 mutation. In the *murA* gene, none of the strains were found to contain the published Mu50 mutation. Mu50, 5 VISA and 8 hVISA had identical sequences to N315. However, 4 VISA and 2 hVISA sequences contained a base substitution (BS) at A897T, which had no affect on amino acid content and was present in the published MW2 sequence. The published Mu50 mutation in odhA was BS at A2526C and DEL at 2651. 6 VISA and 9 hVISA had identical sequences to N315. However, 3 VISA, 1 hVISA and all VSSA plus Mu50 and Mu3 had BS at

A2526C. The 3 VISA and 1 hVISA also had 17 other BSs plus all VSSA had 2 extra BSs. All strains had an extra 66 bp. The 66 bp INS and all BSs were found to be present in MW2. In the *rnr* gene all VISA, hVISA including Mu50 and Mu3 had identical sequences to N315. The published Mu50 mutation in *SA2486* gene was present in Mu50 and Mu3 sequences but not in any clinical VISA or hVISA

Conclusions: The published Mu50 mutations in the 11 genes were not found in any clinical VISA or hVISA. The further mutations found to be present in some genes in the clinical VISA and hVISA would be unlikely to play any role in vancomycin resistance as all were also found in vancomycin sensitive MW2 strain.

P1558 Comparing the gene expression of ABC transporter, protein A and capsule genes between vancomycin sensitive and resistant *S. aureus* strains

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Objectives: This study was to compare the gene expression of three selected genes (ABC transporter, protein A and capsule genes) between vancomycin sensitive and resistant *S. aureus* strains.

Methods: Vancomycin susceptible isolates previously reported and identified to be MRSA (A and C) and their vancomycin-resistant derivatives (B and D) were examined. Vancomycin-resistant derivatives were obtained by Serial passage of the parental strains in nutrient broth with increasing concentrations of vancomycin produced vancomycin-resistant isolates (MIC 8 mg/L). Following RNA isolation using the RNeasy mini kit (Qiagen), RNA fragments (containing the target mRNA) are denatured, separated, then transferred to a sheet of nitrocellulose. The nitrocellulose sheet is hybridized to a single-stranded DIG-labeled DNA probe which will hybridize to the complementary target mRNA and incubated with Anti-DIG-alkaline phosphatase conjugated antibody and substrate for the enzyme is added. Comparing the intensity of the formed bands reflects the amount of specific transcribed mRNA between different *S. aureus* strains, and hence indicates the level of a gene expression.

Results: Northern blotting analysis of the ABC transporter, and protein A genes showed that ABC transporter gene was highly expressed in the vancomycin-resistant strains compared with the sensitive strains while there were no differences in protein A expression between these strains. This study showed that the capsule gene was down regulated in the vancomycin resistant strains.

Conclusions: The up regulation of the ABC transporters may contribute to vancomycin resistance by increasing the transport of the components required for cell-wall synthesis, leading to increased cell wall thickness reported before. The fact that the ABC transporter gene is up regulated in vancomycin resistant strains indicates that it could be targeted therapeutically to develop a vaccine, or antibody therapy. The down regulation of *S. aureus* capsule suggest that the long list of secreted proteins are also down regulated since they are all known to be regulated together by the agr global regulation system. This could explain the significant decrease in the pathogenicity of vancomycin resistant *S. aureus* strains compared with that of sensitive ones in animal work observed.

P1559 Alternative activation of the imipenem resistancegene (cfiA) of *Bacteroides fragilis* among normal microbiota organisms and from clinical infections

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Objectives: Screening for carbapenem resistance among normal microbiota and clinical isolates of *Bacteroides* strains and examination of their resistance mechanisms.

Methods: Fecal samples from healthy individuals were incubated anaerobically in broth cultures containing meropenem (8 and 64 μg/mL) in order to select carbapenem-resistant strains. Such isolates and newly identified carbapenem-resistant *B. fragilis* clinical isolates were examined for the cfiA resistance gene and for the well-known activating insertion sequence (IS) elements by PCR methods and were also examined for production of carbapenemase. The upstream regions of the *cfiA* genes were also amplified by PCR, using one forward primer from the conserved upstream region of cfiA and one reverse primer from the 5′ sequence of cfiA. In one case, the nucleotide sequence of this upstream fragment was also determined.

Results: Seven meropenem-resistant (MICs greater than or equal to 8 μg/mL) *Bacteroides* isolates were selected from 145 fecal samples: 4 *B. fragilis*, 1 *B. vulgatus*, 1 *B. distasonis* and 1 *B. capillosus*. Only the *B. fragilis* isolates harbored the *cfiA* gene. Additionally, 2 carbapenem-resistant *B. fragilis* strains which also harbored the *cfiA* gene were found among clinical samples in Hungary during 2001 and 2002. Interestingly, PCR amplification revealed that none of these *B. fragilis* isolates from normal microbiota and clinical samples carried any IS insertion in their cfiA upstream regions but they all produced a detectable level of carbapenemase. Comparision of the nucleotide sequence of the upstream fragment of one of the *B. fragilis* strains from an infection with that of the upstream region of one cfiA-positive but imipenem-susceptible strain (*B. fragilis* BFr81) strongly suggests that the cfiA gene of this strain is activated by a point mutation in a hypothethical promoter.

Conclusion: The examples of the test strains described here indicate that, in both normal microbiota and in clinical strains, the *cfiA* gene of *B. fragilis* can be up-regulated by an alternative mechanism(s) to IS element activation.

P1560 Discovery of a NmcA Carbapenem-hydrolyzing enzyme in the US Pacific North-west

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Background: Carbapenems are reliably effective in dealing with infections due to organisms producing AmpC and/or ESBL enzymes. In this report we describe an imipenem resistant isolate of *Enterobacter cloacae* recovered from the blood of a leukemic patient. The isolate was found to produce the molecular class A carbapenem-hydrolyzing beta-lactamase, NmcA.

Methods: Isolate's susceptibility was determined by disk diffusion and by Etest. Beta-lactamase production was investigated by analytical isoelectric focusing (IEF), inhibitor studies, hydrolysis assays, and induction assays. The gene encoding NmcA enzyme was amplified by PCR and sequenced using primers designed from the nmcA gene.

Results: The strain was susceptible to piperacillin, piperacillin-tazobactam, cefotaxime, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, gentamicin and trimethoprim-sulphamethoxazole. It was resistant to ampicillin, amoxicillin-clavulanic acid, cefazolin and cefoxitin. By disk diffusion and *E*-test, the zones of inhibition around the carbapenems; imipenem, meropenem and ertapenem were indistinct with inner colonies extending up to the disk or highest concentration on the Etest strip and were interpreted as resistant. Carbapenemase bioassay using a crude enzyme preparation showed evidence of hydrolysis of imipenem, meropenem and ertapenem. IEF confirmed the presence of a clavulanic acid sensitive beta-lactamase with pI of 6.9 which hydrolyzed imipenem 1 μg/mL on IEF overlay. The enzyme was inducible by both cefoxitin and imipenem as demonstrated by disk-induction and attenuated IEF assay. Sequence analysis confirmed the enzyme as NmcA with 100% identity to both structural *nmcA* and regulatory *nmcR* genes.

Conclusion: This is the first report of the detection of the carbapenem-hydrolyzing enzyme, NmcA in North America. While we assume that the observed phenotype was the result of NmcA enzyme, it is possible that the resistance to imipenem resulted from the combined effects of induction of both AmpC and NmcA. The importance of this finding both for clinicians and laboratorians is illustrated by: the ability of the NmcA enzyme to cause resistance to carbapenems; its occurrence in *E. cloacae* (a common nosocomial pathogen); the difficulty in its detection and its inducibility. The continued emergence of novel resistance mechanisms to carbapenems worldwide reemphasizes the need for not only prudent carbapenem use, but also antibiotic use in general.

P1561 Molecular background of multiresistance in a clinical *Escherichia coli* isolate

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Objectives: Recently the rate of acquired antibiotic resistance in *E. coli* has risen. Resistance causes bacteria to grow more slowly due to the additional genetic material they have to replicate and express. Yet the multiresistant strains isolated from hospitals show no such deficits. If bacteria are viable despite so many resistance genes then an evolution of the genes or of their arrangement must have taken place. In order to analyze this evolution the molecular background of such multiresistant strains has to be known. In this

work, we want to elucidate the molecular background of a clinical *E. coli* isolate with multiresistance.
Methods: A representative multiresistant strain of *E. coli* was selected from a group of strains isolated in Berlin hospitals in 2000. Minimum inhibitory concentrations (MICs) of 50 antibiotics were determined. Beta-lactamases were identified by PCR, subsequent sequencing of the beta-lactamase genes, SDS–PAGE and isoelectric focusing. Quinolone resistance was characterized by sequencing *gyrA* and *parC* and the *marOR* region of the mar-operon. Class 1 integrons and Tn21-family transposons were detected by int1 and tnpA PCRs, respectively. Conjugation was performed and resultant transconjugants were analyzed to reveal which resistance markers were transferable. The plasmids of donor and transconjugants were also prepared and analyzed.
Results: MICs revealed resistance to most beta-lactams, quinolones, aminoglycosides, tetracyclines, chloramphenicol and cotrimoxazol. The beta-lactam resistance is caused by the production of a Class C and a Class A beta-lactamase, located on two different transferable plasmids. The quinolone resistance derives from two chromosomal mutations in *gyrA* and one in *parC*. The strain carries genes for at least three different aminoglycoside-modifying enzymes, one nontransferable, two transferable but on different plasmids. Tetracycline, chloramphenicol and cotrimoxazol resistance are all cotransferable. The donor and all transconjugants carry integrons and transposons.
Conclusions: The strain carries at least 11 resistance genes, mostly located on transferable plasmids, conferring resistance to 43 of 50 tested antibiotics. It is remarkable that bacteria with so many resistance genes have a viability similar to their susceptible parent cells. The presence of integrons and transposons allows the bacteria to rearrange these genes and gives them the potential to reduce redundancy and optimize gene expression.

P1562 Conjugation of multiple resistance genes in *E. coli* in a mouse intestine model

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Objectives: *Escherichia coli* is the causal agents of many infections and the most frequent cause of Gram negative bacteraemia. Multiresistant *E. coli* causing infection in humans could originate from production animals through the food chain or it could be transferred from humans. Our previous investigation of clonality among sulphonamide-resistant *E. coli* isolates originating from different production animals as well as human pathogens showed little clonal relation. The purpose of this investigation was to investigate the possibilities of transfer of antibiotic resistance genes in vivo.
Methods: The donor for this transfer experiment originated from a swine production facility and had a resistance profile including ampicillin, apramycin, gentamicin, streptomycin, spectinomycin, sulfonamide, tetracycline, and trimethoprim. The recipient MG1655 showed phenotypic resistance to rifampicin and streptomycin. Transfers were done in a mouse intestine model with streptomycin pretreatment. All transfer experiments were done in vitro before inoculation of the mice and sulfonamide selection were used in all conjugation experiments. Transconjugants, donor and recipient were tested using plasmid profiles, susceptibility tests and were pulsed field typed using the enzyme blnI. PCR was used to reveal the resistance genes.
Results: All results indicated that a conjugative plasmid of approximately 84 kb was transferred to the recipient along with the phenotypic resistance to ampicillin, streptomycin, spectinomycin, sulfonamide and trimethoprim. The transconjugants were isolated in the mouse fecal sample 24 h after inoculation. PFGE and digestion of the plasmid proved the conjugation. We found the conjugative plasmid to contain sulII encoding sulphonamide whereas streptomycin and spectinomycin are expressed from aadA2.
Conclusions: This illustrates that the conjugation of resistance plasmids in vivo is possible in a mouse intestine model. This might suggest the possibilities of resistance gene transfer from an *E. coli* originating from the food chain to another human pathogenic *E. coli* in the intestine. Resistance to ampicillin and sulphonamides are found frequently in pathogenic *E. coli* from humans and the transfer of resistance genes through the food chain might be as important as transfer of pathogenic organisms.

P1563 Effect of chemical agents on plasmid curing of *Escherichia coli* for determination of antibiotic resistance mechanisms

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Plasmids permit their bacterial hosts to survive better in an adverse environment or to compete better with other microorganisms occupying the same ecological niche. The medical importance of plasmids that encode for antibiotic resistance as well as specific virulence traits has been well documented and demonstrated the important role these bacterial genetic elements play in nature. Elimination of plasmid DNA (curing) is an important step in identifying the phenotypic traits encoded by a given plasmid. A multitude of different chemicals including inhibitors of DNA replication, intercalating drugs and bacterial surface agents has been used as curing agents. However, apart from its spontaneous loss very little is known about how to cure the plasmids. Agents that interfere with plasmid replication can result in curing of the plasmid from bacteria cell. Some chemical agents are Mitomycin C, Ethidium bromide, Acridin orange, Acriflavin, SDS. We have classificated the agents and introduce the best curing agents effects on *E. coli* for determination of source of antibiotic resistance. The results obtained demonstrate that at concentration MIC to 1/8MIC curing agents such as mitomycin C(5–60 ng/mL), Ethidium bromide(10–125 μg/mL), Acridin orang (10–150 μg/mL) had curing activity on *E. coli*. The chemical agents are inhibitor of DNA replication. Curing agents such as SDS(0.05–1.5%), PEG (1–8%) and EDTA(0.05–3%) are bacterial surface agent.

P1564 Cefotaximase-M type beta-lactamase production in *Escherichia coli* isolated at a university hospital in Turkey

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Objective: CTX-M producing Enterobacteriaceae have been reported from throughout the world but there has been limited data for Turkey. Beginning from the last 2 months of 2001, *E. coli* producing extended spectrum beta-lactamases (ESBLs) with a cefotaximase phenotype became prevalent in several clinics at our hospital. This prompted us to investigate CTX-M production among these isolates.
Methods: A total of 14 ESBL positive (according to NCCLS criteria) clinical *E. coli* isolates were collected from December 2001 to February 2002. PCR analysis for *bla* CTX-M genes were performed. Isoelectric focusing (IEF) for the beta-lactamases and transfer experiments were also done. DNA sequencing was performed for the identification of the CTX-M type for five selected isolates. Clonal relationship of the isolates were determined by ERIC PCR.
Results: bla genes encoding for CTX-M were amplified in all 14 isolates. DNA sequences were identical to CTX-M-3. IEF revealed two beta-lactamases with pIs of 5.4 and 8.4 which were cotransferred to recipient *E. coli* J53-2. A common plasmid with a molecular size of 68 kb were identified in 11 (78.6%) of the 14 transconjugants. Seven ERIC-PCR patterns were detected among the parental isolates.
Conclusions: (i) CTX-M-type ESBL producing *E. coli* is a rapidly growing problem in our hospital (ii) Both horizontal and vertical gene transfer is responsible from the dissemination of CTX-M type beta-lactamases (iii) This is the first report of CTX-M-3 beta-lactamase producing *E. coli* strains from Turkey.

P1565 Conjugal transfer of aminoglycoside and macrolide resistance among *Enterococcus faecium* in vitro and in vivo in the intestine of mice

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Background: *Enterococcus* spp. is one of the most common bacterial genus encountered in clinical specimens. Enterococci are known to harbor different genetic elements such as conjugative transposons and different types of plasmids with resistance genes. A hot spot for gene transfer could be the

gut of different animal species including humans, since transient colonization of enterococci in the human gut easily takes place after ingestion of food containing such bacteria. The aadE, aphA-3, and erm(B) encoding resistance to streptomycin, kanamycin and erythromycin, respectively, are found on the erm(B)-Tn5405-like element in *Enterococcus faecium*. This study investigated the presence of the erm(B)-Tn5405-like element in a multiresistant human *E. faecium* isolate together with the presence of aac(6′)-Ie-aph(2″)-Ia. The transfer of these genes was also studied both in vitro and in vivo in a mouse model.
Methods: A human *E. faecuim* isolate 160/00 harboring the *erm(B)*, *aac(6′)-Ie-aph(2″)-Ia*, *aadE*, and *sat4* resistance genes, was used as donor. The strain used as recipient was *E. faecuim* 64/3, which is resistant to streptomycin, rifampin, and fusidic acid. Conjugation in vitro was performed by the filter mating procedure. In vivo transfer experiments were carried out in the intestine of streptomycin treated mice.
Results: 160/00 harbored the erm(B)-Tn5405-like element. Co-transfer of erm(B), aadE and aphA-3 genes indicated transfer of this element. Co-transfer of aac(6′)-Ie-aph(2″)-Ia was obtained in all in vivo transconjugants recovered in feces and in 26 out of 30 in vitro transconjugants tested. in vitro frequencies of transfer was approximately 2×10^{-5} transconjugants/donor. 24 h after inoculation, transconjugants harboring the erm(B)-Tn5405-like element and aac(6′)-Ie-aph(2″)-Ia could be recovered in vivo. Throughout the experiment, high numbers of transconjugants approaching 6 log10 CFU/g of feces was observed. Plasmid profiles and Southern blots showed that both the erm(B)-Tn5405-like element and aac(6′)-Ie-aph(2″)-Ia was placed on the same large plasmid (> 150 kb).
Conclusion: These results indicate that the intestine could be a hot spot for gene transfer of resistance genes. Both in vitro and in vivo several resistance genes were transferred at the same time without any selective pressure.

Epidemiology of resistance 6

P1566 Penicillin resistance of *Streptococcus pneumoniae* isolated from community-acquired lower respiratory tract infections in Manisa, Turkey

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Objectives: The increasing frequency of penicillin and multi drug resistant pneumococci all over the world has resulted difficulties in therapy for pneumococcal infections in recent years. It is recommended that resistance surveillance programs routinely should follow the resistance at regional areas and also the findings of resistance fenotypes should be reported. The aim of this study is to investigate the rates of resistance to penicillin and other antimicrobial agents in *Streptococcus pneumonia* strains isolated from community acquired lower respiratory tract infections in Manisa, Turkey.
Methods: Seventy-five strains were assessed in the study and penicillin resistance was evaluated by using oxacillin disc diffusion test and *E*-test methods. Resistance to the other antimicrobial agents were investigated by disc diffusion method.
Results: We found the rates of intermediate resistance and high resistance to penicillin as 8.0% and 2.7%, respectively. The rate of multidrug resistance (MDR) was found as 4.0%. MDR fenotypes, encountered in our region are associated with macrolide and trimethoprim/sulfamethoxazole resistance as well as penicillin resistance. We could not able to find any strain resistant to ofloxacin, vancomycin or imipeneme. The rates of resistance to trimethoprim-sulphamethoxazole, erythromycin, gentamicin, cefaclor, clindamicin, aztreonam, amoxycillin-clavulanate, chloramphenicol and ceftriaxone were found as 18.7, 16.0, 5.3, 5.3, 4.0, 2.7, 2.7, 1.3 and 1.3%, respectively.
Conclusions: As the rates of high penicillin resistance were low in our region, we concluded that penicillin should be the first choice in the management of empirical therapy. But also the surveillance programs monitoring antimicrobial resistance against *Streptococcus pneumoniae* should be performed currently and the findings should be reported to clinicians.

P1567 Oropharyngeal carriage and penicillin resistance of *Neisseria meningitidis* in primary school children in Manisa, Turkey

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Objectives: The mortality and morbidity of epidemic meningitis caused by *Neisseria meningitis* are still high in both developing and industrial countries. The increasing resistance of penicillin which is the first treatment choice has been reported in the recent years. This study aimed to determine the oropharyngeal carriage rates and serogroups of *N. meningitidis* in primary school children in Manisa. Penicillin resistance and resistance mechanisms of isolates were also investigated.
Methods: Oropharyngeal swabs were obtained from 568 children aged 7–14-year-old in this study. The specimens were cultured in GC agar enriched vitox (Oxoid) and Thayer Martin medium (VCNT antibiotic supplement SR91; Oxoid) and the identification was made by biochemical properties using commercial kits (BBL Crystal N/H ID-Becton Dickenson). The serogroups were identified depending on capsular antigens by specific antisera (Difco). Penicillin resistance of strains were investigated by using oxacillin disc diffusion method and *E*-test methods and then Nitrocefin test (Nitrocefin strip, Oxoid) was used to determine the mechanism of resistance.
Results: The results of this study showed that the carriage rate of *N. meningitidis* in our region was 6.3% (36 strains) and the serogroups identified were serogroup C (36.1%), A (27.8%), B (22.2%), W-135 (11.1%) and D (2.8%), respectively. The penicillin resistance was found in eight strains (22.2%), while beta lactamase activity was found in none.
Conclusions: In conclusion, the carriage rate of *N. meningitidis* and serogroups are similar to studies reported from other countries. As penicillin resistant strains are found in our region, the surveillance programs monitoring antimicrobial resistance should performed and the risk groups have to be vaccinated.

P1568 Vancomycin-resistant enteroccoci in Lebanese farms

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Introduction: Resistance against commonly used antibiotics has been observed since these agents were introduced in medicine. In the modern chicken farms antibiotics are used in high quantities not only for treatment and prevention of bacterial diseases, but also as growth promoters (AMGPs). Several studies correlated between the use of antimicrobial agents as growth enhancer and the emergence of resistant bacteria. In this study, our aim was to assess the occurrence of VREs in chicken from different farms in Lebanon.
Materials and methods: This study involves 10 big farms located in the five different Lebanese governerates. The concerned population encompasses 223 chickens, 46 chicken consumers and three farmers. Rectal swabs were obtained and isolation was done on VACC medium (Blood agar added with vancomycin, amphotericin B, clindamycin and ceftazidim). Identification and MICs were determined as indicated by the NCCLS. A multiplex PCR-assay was realized to detect the presence of different genes encoding resistance to glycopeptides
Results: Results are detailed in Table 1. A total of 25 VREs were detected from the 223 samples of chicken, no VRE detected in humans. These 25 VREs contributed to 11% of the total collected samples. The dominant species of VRE was *E. faecium* which contributed to 44% of the total VRE in chicken, whereas, *E. gallinarum* represents 40% of the VRE, *E. avium* contributes 16% of the VRE No *E. faecalis* was isolated.

Table 1 Represents the distribution of different species of VRE with their corresponding phenotypes

	Beirut	Bekaa	Mount Lebanon	North	South	Total
Number of samples	12	74	93	13	31	223
VRE	2	11	6	6	0	25
Van A	2	4	4	2	0	12
Van B	0	3	0	0	0	3
Van C	0	4	2	4	0	10
E. Faecium	2 (*vanA*)	3 (*vanA*), 1 (*vanB*)	3 (*vanA*)	2 (*vanA*)		
E. Faecalis	0	0	0	0	0	0
E. galinarium	0	4(Van C)	2(Van C)	4(Van C)	0	10
E. avium	0	1(A), 2(B)	1(A)	0	0	4

Discussion: Our results represent the first Lebanese data illustrating the actual situation of resistance to vancomycin among Enteroccoci occurring in chicken. This study gives an idea about the occurrence of this highly resistant germ and opens horizons to future studies that should focus on the antibiotics used in the Lebanese farms and their relationship with the emergence of resistant strains. It was not surprising to find the highest percent of resistance in Bekaa due to the wide distribution of farms and to the absence of control and monitoring of antibiotic use. Our data showing the maximum of occurrence of resistance in *E. faecium* meets other authors' data. Van A and Van B Genotypes of VRE, having an acquired type of resistance, indicate that there occurrence is most probably correlated with the use of antibiotics as growth promoters or for therapy.

P1569 Antibiotic resistance in the oral cavity

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Objectives: There are large amounts of data describing the incidence of antibiotic resistance in a number of different clinical settings and for a wide range of microorganisms. However, little attention has been paid to the presence of antibiotic resistance in dental infections. Between January 2001 and January 2002 we conducted a multicenter UK study investigating the antimicrobial susceptibility patterns of microorganisms isolated from acute dento-alveolar abscesses.
Methods: A total of 65 pus aspirates from dento-alveolar abscesses were investigated using routine diagnostic microbiology techniques. Antimicrobial susceptibility testing was performed using BSAC methodology and *E*-test strips.
Results: A total of 263 microbial strains were isolated consisting of 76 (29%) facultative anaerobes and 181 (71%) strict anaerobes. Low levels of resistance were detected to the commonly prescribed dental antibiotics. Penicillin resistance was detected in 3 (1%) of isolates; all of these were found in *Prevotella* spp. Cephalexin resistance was detected in 11 (4%) of isolates. Erythromycin resistance was found in 8 (3%) of isolates, most of which were from the *Fusobacterium* spp. Tetracycline resistance was found in 9 (3%), mainly in the *Prevotella* spp.
Conclusions: The majority of isolates from acute dento-alveolar infections in adults remain susceptible to the antibiotics most commonly prescribed for dental infections.

P1570 Levels of antibiotic resistance in isolates of *E. coli*, *Enterococcus*, *Campylobacter* and *Salmonella* species isolated from pigs and chickens in Hong Kong

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Introduction: Antibiotics are used in animal husbandry for growth promotion, prophylaxis and therapy. There remain considerable concerns that such use may lead to increased resistance to the antibiotics used in human therapy due to cross resistance. Resistance in normal flora organisms may be transferred to other pathogenic species by conjugation and transformation. *E. coli*, *Enterococcus*, *Salmonella* and *Campylobacter* are the bacteria most likely to infect humans from food animals and carry with them antibiotic resistant determinants. This study aimed to determine levels of resistance in isolates of these organisms from pigs and chickens bred in Hong Kong.
Methods: Organisms were isolated using standard methods from chicken respiratory tract, intestine and feces and from liver, trachea, intestines and feces of pigs from livestock farms in HK. Antibiotic sensitivity testing was performed using disc diffusion technique and interpreted according to NCCLS recommendations.
Results: 63 samples yielded *E. coli* with 73% resistant to ampicillin, 98% to tetracycline, 22% to cephalothin, 54% to ciprofloxacin, 76% to cotrimoxazole, 57% to chloramphenicol and 41% to gentamicin. Twenty-three samples yielded Enterococci with little differences in resistance between chicken and pig isolates. Although no high level vancomycin resistance was noted, two chicken isolates showed intermediate levels of resistance. Resistance to aminoglycosides (streptomycin 100%, gentamicin 74%) and tetracycline (100%) was high but to ampicillin was low (4%). Eight samples yielded *Campylobacter* all of which were resistant to ciprofloxacin and tetracycline, 50% to erythromycin. *Salmonella* was isolated from four pig specimens. All were resistant to ampicillin, tetracycline, cotrimoxazole, gentamicin and chloramphenicol. There was no resistance to ciprofloxacin or cephalothin.
Conclusions: High levels of antibiotic and multidrug resistance were observed. Regulations to reduce drug use and to limit choices to those not used in humans have been implemented in some countries. In HK only two substances, chloramphenicol and avoparcin, are prohibited for animal use, though levels at the time of slaughter are monitored for 36 other antibiotics. High levels of chloramphenicol resistance were detected despite its prohibition. The ban on avoparcin use may be related to the absence of high level vancomycin resistant enterococci. More stringent controls on antibiotic use may be appropriate.

P1571 Changing epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Finland

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Background: The prevalence of MRSA amongst invasive *S. aureus* isolates has remained low in Finland. However, the annual number of MRSA isolates reported to the national infectious disease register has constantly increased. In addition, molecular typing has revealed several outbreaks, especially outside Helsinki metropolitan area. To get further insight to these changes, we analyzed the antibiotic resistance profiles of MRSA isolates sent to the national reference laboratory in 1997–2001 and reviewed the diagnostic methods used for MRSA identification in Finnish microbiology laboratories in 2001.
Methods: The proportion of isolates resistant to methicillin only and of those expressing multidrug resistance was estimated based on the disk diffusion test and oxacillin MIC *E*-test values determined at the national reference laboratory. A questionnaire-based survey sent to microbial laboratories assessed, in addition to diagnostic methods used, the number of MRSA screening specimens studied and the total number of positive MRSA findings in 2001. To calculate the rates of MRSA by each 20 hospital districts, data from the national population register was used as a denominator.
Results: Amongst the total of 1114 MRSA isolates sent to the reference laboratory, 33% were multidrug resistant. The proportion of isolates resistant to methicillin only increased from 9.5% in 1997 to 33.6% in 2001; outside Helsinki from 8.8% to 30.6%. Of the isolates, 3% had oxacillin MIC < 2 μg/mL. The NCCLS guidelines for the oxacillin disk diffusion test were, in general, followed, expect for the incubation time, which was only 18 h (instead of 24 h) in 35% of the laboratories. Half of the laboratories used MRSA-Screen(R) test routinely. The number of screening specimens studied varied between 0 and 2768 per 100 000 population by hospital district and the rates of MRSA between 0 and 48 per 100 000 population; in five hospital district >17. The high rate of MRSA did not always relate to a high rate of screening samples taken.
Conclusions: There was a wide geographic variation in the rates of MRSA, but also amongst the screening practices. Non-multidrug resistant MRSA isolates, especially those resistant to methicillin only, showed an emerging trend. Part of these MRSA strains could remain undetected in some laboratories because of current diagnostic techniques used.

P1572 Antibiotic susceptibility and strain typing of animal *Staphylococcus aureus*

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Objectives: Evidence has emerged which strongly indicates that antibiotic use in animals has created a reservoir of resistant bacteria that have spread through the food chain to humans. However, little data is available on *Staphylococcus aureus* from animal. In this study we have looked at antibiotic susceptibility and strain types of *S. aureus* from various animal sources.
Methods: One hundred and forty-four *S. aureus* isolated in animals were screened for resistance against 19 antibiotics by BSAC method (Andrews J.A.C. 2000) and typed by PCR-Ribotyping and PFGE. The *S. aureus* were isolated from different geographical locations; Aberdeen, Northern Ireland, Ayrshire and Inverness and from various sources: milk (53), chicken (37), cow (34), pheasant (6), pig (4), partridge (3), horse (2), Turkey (2) and lamb, parrot and unknown (1 each).
Results: Seventeen distinct resistance phenotypes were detected amongst the 144 isolates tested. 42% were fully sensitive, 27% were resistant to one antibiotic, 19% were resistant to two antibiotics, 8% were resistant to three antibiotics and 4% were resistant to five antibiotics. Resistant to tetracycline (72%) was the most prevalent followed by ciprofloxacin (43%), streptomycin (23%), clindamycin (16%), erythromycin (14%), tylosin (7%), sulphamethoxazole (2%), chloramphenicol (2%), rifampicin, amikacin and tobramycin (1%, respectively). Tetracycline and ciprofloxacin resistance predominated within chicken populations and accounted for 59% of all resistance detected. The 144 isolates were distinguished into 58 PFGE types (defined as isolates with 3 or fewer band differences). Two types, Types A and T, accounted for 17 and 13%, respectively. PFGE Type A was associated with chickens from Northern Ireland and PFGE Type T was associated with cattle from Aberdeen.
Conclusion: Resistance to antibiotics used to treat *S. aureus* in clinical medicine is found in animal *S. aureus*. Further studies are warranted to investigate whether horizontal transfer of these resistance elements from animals to man has occurred.

P1573 Evaluation of two rapid methods for detection of methicillin resistance in *Staphylococcus aureus*

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Objectives: The rapid and accurate identification of methicillin-resistant *S. aureus* (MRSA) is important in order to control nosocomial infections. The purpose of this study was to evaluate the ability of two rapid methods to detect MRSA, and compare them with the PCR detection of the *mecA* gene.
Methods: A total of 103 clinical isolates of *S. aureus* identified by Vitek System 1, were studied by three methods: MRSA-Screen latex agglutination (Innogenetics), Velogene Genomic ID Assay for MRSA (Alexon-Trend) and a PCR-Multiplex assay for the *mecA* and *femA* genes of *S. aureus*.
Results: Of the 103 isolates tested, 61 (59.2%) were resistant to methicillin, and only one tested equivocal by the agglutination method (positivity after 3 min). The others 42 (40.8%) isolates were found to be sensitive to methicillin. Using as reference method the mecA PCR positivity, the Velogene Genomic ID Assay for MRSA (Alexon-Trend) and the MRSA-Screen latex agglutination (Innogenetics) assays showed a sensitivity of 100 and 98%, respectively. There were no false-positive reactions for both methods (100% specificity).
Conclusions: The correlation between the two methods and PCR, suggests their use as screening methods for the rapid detection of MRSA. Despiste the better sensitivity of the Velogene assay, the ease of use, turnaround time and relative cost of MRSA-Screen latex agglutination, are important factors for its selection as the routine method for the MRSA screening in a clinical diagnostic setting.

P1574 Characterization of *S. pneumoniae* and *S. pyogenes* strains isolated from the respiratory tract of pediatric outpatients in Germany, 2002

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Objectives: Respiratory tract infections caused by pneumococci and *S. pyogenes* are serious health problems world-wide. The objective(s) of this research is to study the epidemiological resistance and resistance mechanisms among *S. pneumoniae* and *S. pyogenes* among pediatric outpatients in Germany.
Methods: In a multicenter study including 10 clinical laboratories, a total of 239 *S. pneumoniae* and 236 *S. pyogenes* strains were collected between January through December 2002. Serotyping of pneumococci was done with the quellung reaction. MICs were determined according to NCCLS by microbroth dilution method; resistance phenotypes of both pathogens were determined by disk diffusion test and PCR of macrolide resistant determinants was performed according to standard methods.
Results: Resistance rates of *S. pyogenes* isolates were as follows (intermediate and resistant): Penicillin 0%, cefotaxime 0%, amoxicillin 0%, erythromycin A 14%, clindamycin 0%, gatifloxacin 0% and telithromycin 0%. *S. pneumoniae* isolates showed the following resistance rates (intermediate and resistant): Penicillin 5%, cefotaxime 1.7%, amoxicillin 0.8%, erythromycin 19.7%, clindamycin 7.5% and gatifloxacin 0%. The new ketolide telithromycin was 100% active against all pneumococcal and *S. pyogenes* isolates with MIC50 = 0.016 mg/L and MIC90 = 0.125 mg/L for pneumococci; and MIC50 = 0.03 mg/L and MIC90 = 0.5 mg/L for *S. pyogenes*. Of 47 macrolide-resistant pneumococcal strains 29 (61.7%) were M phenotypes pocessing the mefA resistant determinants and 18 (38.3%) were cMLSB pocessing the ermB resistance determinants. 33 isolates of *S. pyogenes* were macrolide resistant, among which 31 (93.9%) were M phenotypes and 2 (6.1%) strains were inducible (iMLSB).
Conclusions: Macrolide resistance among children is an increasing problem in Germany. Telithromycin was highly active against all pneumococcal and *S. pyogenes* isolates.

P1575 Surveillance of antimicrobial resistance in four major pathogens of community-acquired respiratory tract infections in Germany, 2002

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Objectives: *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, and Moraxella catarrhalis are key pathogens of community-acquired respiratory tract infections (CA-RTI). Resistance to various antibiotics was reported to increase in Germany. The objective of the study was to determine the prevalence of resistance against commonly used antibiotics applying DIN- and NCCLS interpretive criteria.
Methods: Twenty-four microbiology laboratories throughout Germany participated in the study. Strains were sent to a reference laboratory for re-identification and susceptibility testing using the microdilution method according to DIN-standard 58940. Antibiotics tested were Penicillin G (PEN), ampicillin/sulbactam (AMP/SBT), cefuroxime (CEF), erythromycin (ERY), clindamycin (CLI), and levofloxacin (LEV). SBT was tested in combination with AMP at a fixed concentration of 8 mg/L. As there are no recommended NCCLS breakpoints for M. catarrhalis and AMP/SBT for *S. pneumoniae*/*S. pyogenes*, those recommended for *S. aureus* and of AMP for *S. pyogenes*, respectively, were applied. Production of beta-lactamase was detected by nitrocefin. From January to April 2002 a total of 1279 isolates were collected: *S. pneumoniae* ($n = 331$), *S. pyogenes* ($n = 340$), *H. influenzae* ($n = 300$), and M. catarrhalis ($n = 308$).
Results: Susceptibility rates (%) were as follows (DIN/NCCLS): *S. pneumoniae* – PEN (95/97), AMP/SBT (100/98), CEF (98/98), ERY(84/79), CLI (94/93), LEV (–/100); *S. pyogenes* – PEN (100/100), AMP/SBT (100/100),

CEF (100/100), ERY (87/84), CLI (97/97) LEV (–/100); *H. influenzae* – PEN (53/–), AMP/SBT (100/100), CEF (98/100), ERY(1/–), LEV (–/100); M. catarrhalis – PEN (< 1/ < 1), AMP/SBT (100/100), CEF (67/99), ERY (>99/>99), CLI (34/11), LEV (–/100). The rate of beta-lactamase producing strains was 8.7% for *H. influenzae*, while that for *M. catarrhalis* was 99.3%.
Conclusions: Our results show that isolates of *S. pneumoniae* were more susceptible to PEN than to ERY and that isolates of *S. pyogenes* were more susceptible to ERY than those of *S. pneumoniae*. Comparing DIN and NCCLS breakpoints, susceptibility rates of *S. pneumoniae* and *S. pyogenes* for ERY according to DIN were higher than those according to NCCLS. AMP/SBT and LEV showed the highest in vitro activities against the four key pathogens causing CA-RTI

P1576 Phenotypic and molecular typing of methicillin-resistant *Staphylococcus aureus* strains isolated in an Italian hospital

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the most important pathogens. In Italy the frequency of isolation of MRSA in nosocomial environment is nearly 35%. Recent reports indicate that the therapeutic options available for invasive MRSA infections are decreased by the isolation of strains with reduced susceptibility to glycopeptides. The aim of the present study was to investigate the antibiotic susceptibility of MRSA in our hospital.
Methods: 120 MRSA were collected from different hospital wards (Jan – April 2001). Standard microbiological methods for identification of *S. aureus* included Gram stain, growth in mannitol salt agar, coagulase test, API ID32 Staph. Methicillin-resistance was screened by disk diffusion test in Mueller Hinton agar with oxacillin. The presence of *mecA* gene was determined by PCR. Antimicrobial susceptibility tests were performed by microdilution method and the antibiotics were cefazolin (CEF), erythromycin (ERY), gentamicin (GEN), rifampin (RIF) ciprofloxacin (CIP), tetracycline (TET), fosfomycin (FOS), vancomycin (VAN), teicoplanin (TEC), trimethoprim/sulphamethoxazole (TSX). Molecular analysis was performed by *Sma*I-pulsed field gel electrophoresis (PFGE).
Results: The majority of MRSA were isolated from patients admitted to surgical wards (61.6%) and intensive care units (30%). All MRSA contained *mecA* gene. 15% of strains were resistant only to beta-lactams, but the majority were multiresistant. All isolates were susceptible to vancomycin but two strains were intermediate to teicoplanin (MIC = 16 mg/L) (TISA). Eleven resistant phenotypes (antibiotype) were found and 61.6% of strains examined displayed the same antibiotype. Strains with the same antibiotype exhibited more then one PFGE type.
Conclusion: In countries such as Italy, in which teicoplanin has been introduced in clinical medicine many years ago, it is important to identify TISA. These isolates are not readily detected by disc diffusion method so as much information as possible should be collected to clarify their role in therapeutic failure.

P1577 STAR elements and antibiotic resistance in *S. aureus*

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Objectives: *Staphylococcus aureus* causes many types of human disease and, in particular, is the most common bacterium cultured from infected wounds. There are seven publicly available genome sequences and much is known about its molecular biology. There is increasing effort to tie molecular knowledge to improvements in clinical practice, with interest in ways to predict virulence or establish strain relatedness. The *Staphylococcus aureus* repeat element (STAR) is a variable length GC-rich region present in multiple copies (<100) on the *S. aureus* genome. It is highly polymorphic and has potential as an epidemiological marker. The goal of this study was to determine if STAR patterns could be used to group strains and predict antibiotic resistance. We hypothesized that related strains would have the same STAR pattern and similar resistance.
Methods: In total, 64 different *S. aureus* strains were investigated. Forty-five strains were isolated from wound cultures, five from central venous catheters of ICU patients, and 14 were common laboratory control strains. The subject population consisted of patients treated at the University of Tübingen (Germany) hospitals and clinics. Strains were isolated in the course of routine clinical diagnostics. Investigations consisted of assessment of antibiotic resistance at concentrations greater than 5 μg/mL and PCR for STAR elements at two intergenic regions.
Results: Please see table for results.

STAR Pattern	Total Strains	% Resistance: Methicillin	Tetracycline	Erytrhomycin	Gallidermin	None
III/IV	8	100*	13	100*	38*	0*
II/I	7	57*	0	14	0	43
IV/II	14	7	21	0	0	29
III/I	7	0	14	0	0	86
III/II	5	0	0	0	0	80
IV/I	6	0	17	50*	0	33
VI/II	5	0	20	20	0	20
Other	12	0	8	17	8	42
Total	64	20	13	23	6	39

*$P < 0.05$ compared to other STAR patterns for the same antibiotic.

Conclusions: There were seven groups of five or more strains with the same STAR pattern. Twelve strains exhibited other STAR patterns. Methicillin resistance was concentrated into two groups. The group with STAR pattern III/IV had high rates of antibiotic resistance, with every strain resistant to at least one antibiotic. There were two control strains, two ICU isolates, and four wound isolates in this group. STAR pattern II/I also exhibited methicillin-resistance, but consisted of seven wound isolates collected from three patients. The remaining STAR groups exhibited variable amounts of resistance. This study shows that the STAR element is useful for grouping strains and may aid in predicting resistance.

P1578 Use of the International Circumpolar Surveillance System for population-based surveillance of invasive pneumococcal disease 1999–2001

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Background: The International Circumpolar Surveillance (ICS) Project is an infectious disease surveillance network of hospital and public health laboratories working together with public health practitioners in Arctic countries. In 1999, surveillance for invasive disease caused by *Streptococcus pneumoniae* (Sp) began in the US Arctic, Alaska (AK) and Northern Canada (N Can), then expanded in 2000 to include Greenland (GN), Iceland (IC), Norway (Nor) and Finland (Fin).
Methods: We defined a case of invasive Sp as an individual from whom pneumococci were isolated from a normally sterile site. We analyzed ICS data on invasive Sp disease from January 1999-December 2001 to determine: (i) Common clinical syndromes and risk factors; (ii) Rates of disease by country/region; (iii) serotype distribution; and (iv) Antimicrobial susceptibility patterns. All available isolates were serotyped; antimicrobial susceptibility testing was done by microbroth dilution (AK and N Can), agar dilution (GN and Fin), or disc diffusion (IC and Nor).
Results: A total of 3554 cases of laboratory confirmed invasive pneumococcal disease were reported from AK (343), N Can (133), GN (11), IC (79), Nor (1729), and Fin (1259). Ethnicity and risk factor data were not available from Nor or Fin. Serotype data was not available from Nor. Overall, case-fatality ratios varied by country (range: 3.8–18.2%). Pneumonia (46%), septicemia (32%), and meningitis (8%) were the most commonly reported clinical presentations. Smoking was the most commonly reported risk factor in persons >18 years of age (37%). Crude annual rates of invasive Sp disease per 100 000 population varied by country (range: 9.7–33.2). Rates of invasive Sp disease among native (aboriginal) persons in AK and N Can were 42 and 43 cases per 100 000 persons, respectively. The highest rates of Sp disease occurred among children < 2 years of age (range: 36–184/100 000). Overall, 72% of Sp isolates from children < 2 years of age were serotypes contained in the 7-valent vaccine. Among isolates from persons >2 years of age, overall, 88% were serotypes contained in the 23-valent polysaccharide vaccine. The proportion of isolates fully resistant to penicillin varied by country from <1% in Fin to 10% in AK.

Conclusions: Rates of invasive pneumococcal disease are high in natives and children <2 residing in Arctic countries. Case fatality from invasive disease appears higher in some countries, warranting further investigation.

P1579 International Inter-Laboratory Quality Control program for Circumpolar Surveillance of *Streptococcus pneumonia*e

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Background: In 1999, the International Circumpolar Surveillance (ICS) of invasive diseases caused by *Streptococcus pneumoniae* (Sp) was established in the US Arctic (Alaska) and northern Canada(NC). Currently 23 and 14 clinical laboratories in Alaska (AK) and NC forward isolates from patients with invasive pneumococcal disease to reference laboratories in AK and Canada, respectively. To ensure interlaboratory comparability of Sp serotype and antimicrobial susceptibility between two reference laboratories in Canada (Alberta and Quebec) and one in the US (AK) we established the ICS Sp interlaboratory QC program.
Methods: Each reference laboratory was responsible for the export of one QC panel of seven Sp isolates each year to each of the other laboratories using a transportation medium of their choice. Serotyping was performed by Quellung reaction. The antisera inventory included grouping and factoring antisera for the most common serogroups, including 6, 9, 18, 19, and 23. Minimum inhibitory concentration (MIC) was determined for each QC isolate and for ATCC strain 49 619 for those antibiotics which were routinely tested in each laboratory. MIC results for each laboratory were expected to be within one log2 dilution of each other regardless of testing method. Discrepancies of results were documented and examined to determine causes and solutions.
Results: Between January 1999 and December 2002, a total of 12 QC panels of seven Sp isolates each were shipped and tested by all three reference laboratories. Serotyping correlation for 84 isolates was 97%. Discrepancies between serotyping results was attributed to loss of capsule following multiple passing. Overall correlation of the MIC results, within ± 1 log2 dilution was 96.8%. Discrepancies among MIC's between laboratories could be explained by testing method (i.e agar dilution vs. broth dilution) and incubation conditions (i.e with or without CO_2). Transportation times for QC panels between the participating laboratories were generally excellent (2–3 days).
Conclusion: Establishment of an international QC program for Sp between reference laboratories in the US and Canada is feasible. Expansion of the QC program to include reference laboratories in other circumpolar countries should be considered. Challenges include the maintenance of infectious agent specific importation permits, adherence to international transportation regulations, and cost of international shipping of infectious agents.

P1580 Detection of OXA-40 carbapememase in an *Acinetobacter baumannii* epidemic strain from Portugal

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Objectives: Thirty-eight imipenem-resistant *Acinetobacter baumannii* (Ab) clinical isolates from four different hospitals in Portugal were investigated in order to characterize the mechanism of carbapenem resistance and to determine their clonal relationship.
Methods: Susceptibility tests were performed by using the agar diffusion method and MICs of beta-lactams were determined by the *E*-test method. Carbapenemase activity was determined in crude extracts by spectrophotometry using as substrate an imipenem solution (0.1 mM) in the absence and presence of zinc or EDTA. Gene detection was performed by PCR with the PCR SuperMix (Gibco BRL) and blaOXA-24 primers (300 mM).
Pcr conditions were: initial desnaturation of 94°C/3 min, followed by 30 cycles of 94°C/1 min, 50°C/1 min and 72°C/2 min with a final extension of 72°C/8 min. Amplicons were cloned onto pPCR-Script Cam SK(+) vector using the PCR-ScriptTM Cam Cloning Kit (Stratagene). Recombinant plasmids were transformed on Epicurian Coli XL10-Gold Kan competent cells. Nucleotide sequence was analyzed using Clustal W and compared with known sequences. The epidemiological relationship among the isolates was assessed by RAPD using the M13 primer, REP-PCR and macrorestriction of chromossomal DNA with ApaI enzyme followed by PFGE.
Results: All the isolates were highly resistant to penicillins, broad spectrum cephalosporins, aztreonam (MICs > 256 mg/L) and imipenem (MIC > 32 mg/L). They were also resistant to ciprofloxacin and aminoglycosides, except to tobramycin. Extracts hydrolyzed moderately imipenem (specific activity of 5 mU/mg protein). No significant differences were observed in the presence of Zn and EDTA. After sequencing, the deduced protein showed the typical motifs of class D OXA-beta-lactamases and was identified as an OXA-40. DNA fingerprints obtained by RAPD and REP-PCR were identical. Patterns obtained by PFGE were identical or differed in only one or two bands.
Conclusions: For the first time in Portugal, it was detected a class D carbapenemase, OXA-40, a recently described carbapenemase and associated with imipenem resistance. The high incidence of imipenem resistance among Portuguese Ab isolates was mainly due to an epidemic strain disseminated in various hospitals.

P1581 Improved tracking of epidemic methicillin-resistant *Staphylococcus aureus* in Scotland using a combination of pulsed field gel electrophoresis and mec-associated drug sequences

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Background: Two epidemic methicillin-resistant *Staphylococcus aureus* strains, EMRSA-15 and EMRSA-16, first observed in England in the early 1990s, had spread to Scotland by mid 1990. The introduction of these epidemic strains was followed by a rapid rise in reports of MRSA in Scotland from 565 in 1995 to over 12 203 in 2001. EMRSA-15 and −16 account for 70% and 20% of all Scottish MRSA, respectively. PFGE typing has identified 150 and 200 clonal variants of EMRSA15 and EMRSA16, respectively. However, 50% and 35% of EMRSA15 and EMRSA16, respectively, are indistinguishable by PFGE typing thus complicating epidemiological monitoring. We evaluated the potential usefulness of PFGE combined with the analysis of mec-associated dru sequences as a more sensitive approach to tracking MRSA isolates in Scotland.
Methods: EMRSA-16 and -15 were collected from hospitals throughout Scotland. The isolates were initially characterized by macro-restriction (*Sma*I) analysis using PFGE. In addition, mec-associated dru sequences were also analyzed by electrophoresis of intact or *Alu*I-digested PCR products and by direct sequencing.
Results: By PFGE, 90% of EMRSA-15 isolates exhibited one of six patterns (15a,b,c,d,e, or i), of which 50% were PFGE type 15a. For EMRSA-16 isolates, 79% exhibited one of five patterns (16a,b,d,m, or p) of which 35% were PFGE type 16a. Analysis of dru sequences allowed further separation of isolates (including majority types 15a and 16a) into specific subtypes originating from different hospitals and/or geographic regions. This was initially accomplished by a simple comparison of dru PCR product sizes (either intact or AluI digested) which differed depending on the number of 40-bp tandem repeats. Direct sequencing of the dru region was employed to characterize (and in some instances further subtype) isolates that were otherwise indistinguishable.
Conclusions: A combination of PFGE and dru-sequence analysis appears very promising for the epidemiological tracking of epidemic MRSA isolates in Scotland, including strains with apparently highly conserved genetic backgrounds. This approach may have the potential to identify and monitor the movement of specific epidemic MRSA within and between hospitals which would greatly assist public health and infection control efforts to control the spread of these problem organisms.

P1582 Antibiotic resistance surveillance on the basis of high quality routine data: GENARS – German Network for Antimicrobial Resistance Surveillance

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Objectives: Worldwide every day billions of susceptibility tests for human pathogens are performed for diagnostic reasons. Unfortunately these data are not sufficient for surveillance studies because of the lack of comparability, due to methodological variability. The six laboratories of the GENARS-project measure MIC's of about 30 antibiotics. Methods are standardized and quality controlled. This paper describes the reproducibility of the results of the QC

strains and in addition the quality control by the comparison of MIC-distributions of clinical isolates.
Methods: In each laboratory involved in the GENARS-project, all clinical isolates are identified to species level. Generally, all isolates are tested against 32 different antibiotics. In contrast to usual QC strain evaluation we look for those data, where the MIC's of the strains are within the range of the concentrations tested, which increases the sensitivity of the method. The second quality control measure is the comparison of MIC-distributions of clinical isolates, where the modal value of the sensitive population of one species should be identical for laboratories involved.
Results: Evaluation of QC-strain measurements gives a good impression of the quality of laboratory work. For the first half of 2002 more than 130 tests for each QC-strain and antibiotic were evaluated. Some examples are shown in Table 1, demonstrating that the reproducibility of the test modal value ± one dilution step is close to 100% with few exceptions. The second tool to demonstrate reproducibility of test results is shown in Fig. 1. The MIC-distribution for cefuroxime with *E. coli* demonstrates, that the naturally sensitive population peaks at 4 mg/L.

Table 1 Percent of tests which are in the range of ± one dilution step of the modal value of all results

	Ampicillin	Piperacillin	Teicoplanin	Ciprofloxacin	Levofloxacin	Meropenem	Erythromycin
E. coli (ATCC 25922)	94.8	97.7	–	–	–	–	–
P. aeruginosa (ATCC 27853)	–	92.4	–	93.9	99.2	99.2	
E. faecalis (ATCC 29212)	97.7	–	99.2	98.1	100	100	97.7
S. aureus (ATCC 29213)	71.8	–	95.5	–	–	–	72.7

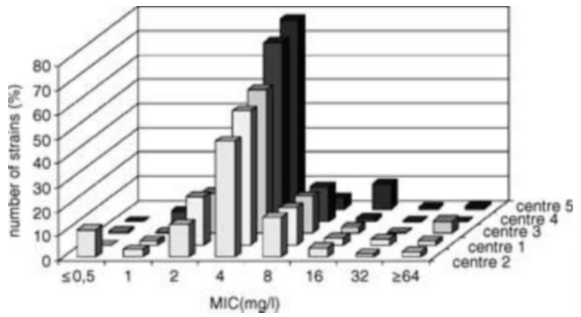

Figure 1 Distribution of MIC-values of E. coli and cefuroximine for five laboratories.

Conclusions: The high methodological standard of sensitivity testing of the GENARS-project will not only improve the surveillance including an early warning system, a demonstration of resistance trends and finding of new resistance mechanisms, but will also be advantageous for the patients who benefit from more reliable test results and better hospital epidemiology.

P1583 Identifying the SCCmec types of MRSA – a comparison of three primer sets

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Objectives: The genetic elements associated with methicillin resistance in *Staphylococcus aureus* have been designated SCCmec (Staphylococcal Chromosomal Cassette mec). Four SCCmec types and three subtypes have been defined, ranging in size between 21 and 67 kb. In this study we compared the effectiveness of three different primer sets (WM, OL, and OH) for identifying the SCCmec types of various MRSA clones.
Methods: Sixty-six MRSA isolates from UK, including representatives of 31 PFGE defined clones, were analyzed using two multiplex SCCmec PCR assays, the WM assay (Morrison et al. Proceedings of the 10th ISSSI, Japan, 2002, Abs. 212–01) and the OL assay (Oliveira et al. Antimicrob. Agents Chemother. 2002; 46: 2155–2161). Isolates with discrepant results were tested with a third set of primers, OH (Okuma et al. J. Clin. Microbiol. 2002: 40; 4289–4294), which involved five separate PCR reactions per isolate.
Results: A total of 45 of 66 (68%) isolates yielded identical SCCmec results with both the WM and OL assays; 2 and 3 isolates were nontypable with the WM and OL primers, respectively; and 1 and 10 isolates, respectively, gave 'new' (presently undefined) typing patterns. Nine isolates gave discrepant SCCmec typing results with the two primer sets. When tested with a third primer set, the OH primers, the SCCmec results for 3 of 9 isolates agreed with those obtained with the WM assay, 2 isolates gave results that differed from those of both other assays, and 4 isolates were nontypable, suggesting the presence of further undefined SCCmec types or subtypes.
Conclusions: It is inevitable that primers directed against different sequences on the large mec DNA inserts of MRSA will sometimes generate different results. International standardization of SCCmec typing assays, and of the definitions and nomenclature for types and subtypes is essential for meaningful comparison of data at the global level.

P1584 Erythromycin-resistant *Streptococcus pyogenes* in the Florence area: at last a stable incidence?

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Objectives: *Streptococcus pyogenes* (SP) is by far the most common agent of acute bacterial tonsillo-pharyngitis (ABTP), as well as an increasingly important cause of invasive infections. Macrolides are frequently used to treat SP ABTP, even in patients who are not allergic to penicillin. The worldwide emergence of erythromycin-resistant (ER) SP strains, primarily in Southern Europe and the Far East, prompted us to evaluate the incidence of ER SP isolates in the Florence area during a 9-year period.
Methods: Between 1 January 1993 and 31 December 2001, we isolated a total of 1294 SP strains from nonconsecutive patients with signs and symptoms of ABTP (1140 throat swabs) or invasive infections (154 skin or blood cultures). Commercial latex agglutination tests were used for rapid identification of SP isolates, which were subsequently tested for susceptibility to penicillin and erythromycin by the disk diffusion method according to the Kirby–Bauer technique. Statistical analysis was carried out by the Chi-square and Fisher exact tests whenever appropriate.
Results: All strains were susceptible to penicillin. During the study period, the rate of all SP isolates that were resistant or intermediate to erythromycin steadily increased from 5% in 1993 to 47.7% in 1999, then it fell to 38% in 2000 and to 34.7% in 2001, with a global figure of 35.1%. A very similar trend was observed for strains isolated from throat swabs, among which the percentage of nonsusceptible SP isolates steadily increased from 5.4% in 1993 to 47.9% in 1999, then it fell to 39.4% in 2000 and to 35.3% in 2001. On the other part, a significantly ($P = 0.006$) lower percentage of strains from other sites were not susceptible to erythromycin (25.3%); moreover, the yearly incidence was more irregular, varying from 0 in the period 1993–1995 to 44.4% in 1999.
Conclusion: The increased incidence of SP isolates that are not susceptible to erythromycin has currently become a serious problem worldwide. In our area, preliminary data indicates that the use of macrolides (in particular, the newer azithromycin and clarithromycin) rose in the early 1990s and subsequently it kept stable or slightly decreased, therefore, it is possible that the worrisome trend in ER SP rate we observed, correlates with macrolide consumption. We conclude that ongoing epidemiological monitoring of ER SP is desirable, and use of macrolides, primarily for respiratory tract infections, must be weighed carefully.

P1585 Molecular epidemiology of vancomycin-resistant enterococci circulating in a large Italian hospital during the last 13 years

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Objectives: In the last decade, vancomycin-resistant enterococci (VRE) emerged as important nosocomial pathogens in several countries, especially in the USA. In Italy the isolation of VRE is an uncommon finding. However during last years at our university hospital an increase of VRE prevalence has been experienced (from 0.1% in 1991 to 3.1% in 2002). In this study, VRE circulating in a large Italian hospital during the period 1991–2002 have been phenotypically and genotypically characterized.
Methods: Fifty VRE (38 *E. faecalis* and 12 *E. faecium*) isolated from various clinical specimens have been studied. Antimicrobial susceptibilities were determined according to the NCCLS (2002) by broth microdilution method. PCR amplification of *vanA* and *vanB* genes was carried out as described by Dutka-Malen et al. (JCM, 1995). Pulsed-field gel electrophoresis (PFGE) was performed as previously described by Miranda et al. (JCM, 1991).

Results: All strains proved to have *vanA* phenotype and genotype. PFGE profiles showed high heterogeneity among the 12 *E. faecium* (10 different patterns). Conversely, among *E. faecalis*, 16 out of 38, belonged to the same PFGE (profile A) and they had the some biochemical profile and antibiotic susceptibility pattern (resistant to erythromycin, ciprofloxacin, tetracycline and fosfomycin simultaneously). 14 were correlated to the profile A and the remaining of isolates (8) showed different patterns. The 16 identical strains were isolated in 4 different wards from the following samples: urine (11), blood (1), vesical catheter (2) and bile (2).
Conclusions: The present results indicate the circulation of a clone of *E. faecalis* characterized by the possession of a great ability to spread in our hospital. Increasing prevalence of VRE observed can be ascribed to the spread of this clone. The presence of some nonclonal strains may be explained by the transfer of mobile genetic elements coding for the *vanA* resistance genotype to unrelated strains of endogenous enterococci.

P1586 Failure of treatment with ceftazidime for infection by *Klebsiella oxytoca* overproducing chromosomal beta-lactamase OXY-1

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Objectives: To establish the reasons for failure of treatment with ceftazidime (CAZ) for infection by *K. oxytoca* overproducing chromosomal beta-lactamase OXY-1 (Ko OXY-1).
Materials: We discuss the case of a patient who in 2002 developed an abdominal infection caused by Ko OXY-1 following traumatic contusion of the tail of pancreas. 10 days of treatment with CAZ (8 g in continuous infusion over 24 h; serum CAZ concentration: 55 mg/L) failed to eradicate the infection (drainage fluids were consistently positive for Ko OXY-1). Successful bacteriologic and clinical recovery was achieved only on treatment with imipenem (IMI) (1 g × 4/24 h; serum IMI concentrations: 16 mg/L [peak] and 2.5 mg/L [trough]).
Results: The strain of Ko OXY-1 involved exhibited an MIC on dilution in gelose of 0.5 mg/L for CAZ and of 8 mg/L for aztreonam (AZT). The MICs for CAZ and AZT with 2 mg/L clavulanic acid (CA) were, respectively, 0.25 mg/L and 0.12 mg/L. The MIC of IMI was 0.5 mg/L both with and without CA. The MICs of amoxicillin, cephalotine, cefuroxime, cefotaxime and ceftriaxone were, respectively (no CA/with 2 mg/L CA: >512/128, 64/2, 32/2, 0.12/0.015 mg/L and 0.12/0.012 mg/L. The enzymatic activity of OXY-1 penicillinase was 100 times superior to that of the activity of a non OXY-1 penicillinase-producing strain and the isoelectric point of beta-lactamase was 7.5. Kinetic analysis of bactericidal effects showed bactericidal activity (decrease in inoculum of 4 log10) within 24 h for 16 mg/L (32 × MIC) of CAZ and within 6 h for 2 mg/L (4 × MIC) of IMI.
Conclusion: These results could account for the therapeutic failure of CAZ and the efficacy of IMI. Poor diffusion of CAZ at the infection site is a further possible contributing factor. This strain is considered sensitive to CAZ in terms of the criteria established by the Antibiogram Committee of the French Microbiology Society in 2002; nevertheless, in view of these results it would appear preferable not to use CAZ for treatment of the Ko OXY-1 strain. Further studies are required.

Infection in the intensive care unit

P1587 Nosocomial bacteremia in the intensive care unit: a case-controlled study of risk factors and outcomes

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Objectives: A case-control study was conducted to identify the risk factors (RFs) associated with nosocomial bacteremia in Intensive Care Unit (ICU).
Methods: During 15 months, 139 patients with bacteremia and 139 control patients in ICU were included in this study. Every patient was matched in a 1 : 1 ratio with controls. Patients who met the nosocomial infection definitions of CDC were included in this study. Frequency, percentage, ratio and categorical variables were analyzed with a chi-square test. Student *t*-test and Mann–Whitney *U*-test were performed for analyzing continuous variables. Multivariate analysis was conducted with a logistic regression.
Results: Two hundred and seventy-eight patients were enrolled in this study. There were 174 bacteremic episodes in 139 patients with bacteremia; 109 episodes by Gram-negative bacilli; 62 episodes by Gram-positive cocci; and 3 episodes by *Candida* spp. *K. pneumoniae* (21.8%), MRSA (20.7%), *P. aeruginosa* (16.1%), *E. coli* (11.5%) were the most common agents in bacteremia. Nosocomial pneumonia (31%), primary bloodstream infections (25.8%) and surgical site infections (9.7%) were the most common infections causing bacteremia. According to univariate analysis, RFs associated with bacteremia were the requirement of mechanical ventilation (MV) more than 7 days ($P = 0.004$), operation ($P = 0.015$), hemofiltration ($P = 0.006$), requirement of total parenteral nutrition (TPN) ($P < 0.001$) and inotropic drug (ID) use on admission ($P < 0.001$) and bronchodilator drug (BD) ($P = 0.041$), previous antibiotic (PA) use ($P < 0.001$). The mean values of AST, ALT, creatinine, leukocyte level and APACHE II and SOFA scores in bacteremic patients were found significantly higher than those of control group ($P < 0.001$ for all variables). The mean values of Hb, platelet count and Glasgow coma score in bacteremic patients were significantly lower than those of the control ($P = 0.002$, $P = 0.038$, $P = 0.017$, respectively). Multivariate analysis revealed that increased creatinine level [Odds ratio (OR) 4.4, $P < 0.001$)], requirement of ID (OR 3.3, $P = 0.004$) on admission, operation (OR 2.1, $P = 0.022$), PA use (OR 1.8, $P = 0.034$), TPN (OR 5.7, $P = 0.001$) could independently be associated with bacteremia in ICU.
Conclusions: Requirement of MV more than 7 days, hemofiltration and BD were found remarkable RFs for bacteremia in ICU.

P1588 Risk factors for mortality of nosocomial bacteremia in intensive care units

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Objectives: The aim of this study was to prospectively follow critically ill patients in ICU to determine risk factors of mortality and outcome associated with nosocomial bacteremia (NB).
Methods: This study was conducted in Intensive Care Units (ICU) (including emergency and general ICU) with 23-bed during 15 months. The risk factors for mortality in 176 patients with bacteremia in ICU were investigated. A chi-square test was employed to compare categorical variables, Student *t*-test and Mann–Whitney *U*-test were performed for analyzing continuous variables. Multivariate analysis was performed with logistic regression.
Results: Two hundred and fourteen bacteremia episodes were found in 176 patients [64 F, 112 M; 51.3 ± 21.3 years-old]: 93 of them died and 83 survived. There were 125 bacteremic episodes by Gram-negative bacilli, and 82 episodes by Gram-positive cocci, 7 episodes by *Candida* spp. in patients with NB. *K. pneumoniae* (21.5%), MRSA (21.5%), *P. aeruginosa* (14.9%), *E. coli* (9.3%) were the most common etiologic agents in bacteremia. Malign hypertension ($P = 0.01$), requirement of mechanical ventilation (MV) more than 7 days, prolonged CVC using time ($P = 0.019$) ($P < 0.001$), having a hematologic malignancy or solid tumor ($P = 0.002$), increased creatinine level (> 1.4 mg/dL) ($P = 0.005$), hemofiltration ($P = 0.007$), requirement of total parenteral nutrition (TPN) ($P < 0.001$) and inotropic drug (ID) on admission ($P < 0.001$) were determined as risk factors for mortality in patients with bacteremia. Multivariate analysis showed that requirement of MV more than 7 days [Odds ratio (OR): 6.8, $P < 0.001$], requirement of TPN [OR: 3.1, $P = 0.034$], requirement of ID [OR: 12.8, $P < 0.001$], increased creatinine level [OR: 2.7, $P = 0.034$] were independent risk factors for mortality of NB in ICU. The mean values of APACHE II scores, SOFA scores and Glasgow coma scale in surviving patients with bacteremia were found significantly different than those of other patients ($P < 0.001$ for all variables).
Conclusions: Although there might be many other mortality causes such as underlying diseases and nosocomial infections in ICU, requirement of MV more than 7 days, requirement of TPN, and ID on admission and increased creatinine level were independent risk factors for mortality in patients with NB in ICU.

P1589 Epidemiology of nosocomial infections acquired in an intensive care unit in Italy

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Objective: To assess the epidemiology and etiology of nosocomial infections (NI) and to identify patterns of antibiotic resistance in a medical-surgical intensive care unit (ICU).
Methods: From April 2000 to April 2002 we conducted a prospective study and detected the most frequent NI and risk factors in patients admitted for more than 48 h to the ICU of the University General Hospital of Verona, Italy. We also evaluated antimicrobial resistance of isolated microorganisms.
Results: During the 2-year survey, 797 patients were evaluated. Median ICU stay was 8 days (range 3–151) and total patient-days were 10.941. Mean age was 64 (SD 19) years and mean APACHE II score 18 (SD 8). Crude mortality was 15%. Invasive procedures were adopted as follows: urinary catheter in 94% of cases, mechanical ventilation in 80%, arterial catheter in 76%, central venous catheter (CVC) in 71%. We recorded 234 episodes (21.4/1.000 patient-days) of ICU-acquired infections in 142 patients (18%). Bloodstream infections (BSI) were the most frequently reported type of infection (141 episodes, 60% of all cases of infection), followed by pneumonia (48 episodes, 21%), urinary tract infections (25 episodes, 11%), wound infections (17 episodes, 8%). Overall the most common pathogens were coagulase-negative *Staphylococci* (CNS) (25%), *P. aeruginosa* (22%), *Enterobacteriaceae* (18%), *S. aureus* (17%), *Enterococcus* spp. (8%), *Candida* spp. (6%), others (4%). Methicillin resistance (MR) was 81% among *S. aureus* (MRSA) and 75% among CNS. Multi-drug resistance was detected in 14% of *P. aeruginosa* isolates and fully resistant strains increased from 2 to 14 from the first through the second year. ICU stay >7 days, CVC retention for >10 days, mechanical ventilation and tracheotomy resulted as risk factors independently associated with ICU-acquired infections.
Conclusions: Overall we detected an ICU-acquired infection rate comparable to other European studies. Nevertheless we observed a different frequency of distribution of NI; in fact BSI were the predominant type of infection. Staphylococci were the most frequently isolated pathogens, with very high levels of MR. We are therefore focussing interventions on staff education about infection control measures (especially catheter management) and on identification and treatment of patients colonized by MRSA. Knowledge about antimicrobial resistance patterns has been very useful to implement periodic antibiotic changes in empiric therapy protocols.

P1590 Prevalence of microbiologic species in intensive care units in Piedmont (Italy)

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Objectives: To assess the principal isolates in Piedmont ICUs and to investigate the main isolation sites and antibiotical resistance.
Methods: A one-day-point-prevalence study was designed and 21 ICUs were enrolled (19 nonspecialized and 2 specialized ones). On the study day biologic samples (for microbiologic survey or etiologic diagnosis) were collected for all the hospitalized patients. For each sample the cultural test was done and, if positive, the antibiogram was performed.
Results: Overall, we performed 241 microbiologic tests: 103 bronchoaspirations (70 positives, 33 negatives), 77 urocultures (28 positives, 49 negatives), 43 emocultures (14 positives, 29 negatives) and 18 CVCs cultures (12 positives, 6 negatives). The main isolates were as follows: *Enterobacteriaceae* ($n = 42$), *Staphylococci* (30 *S aureus*, 11 CNS), *Pseudomonaceae* (28 *P. aeruginosa*, 2 *B. cepacia* and 1 *Stenotrophomonas maltophilia*) and 6 *E. faecalis*. There were also 21 fungal isolates: 15 *Candida albicans*, 4 *Candida nonalbicans* and 2 *A. fumigatus*. The principal isolation sites were summarized in Table 1, while the antibiotic resistance is shown in Table 2.

Table 1 Principal isolation sites

	Enterobacteriaceae	*Staphylococci*	*Pseudomonaceae*	*E. faecalis*	*Mycetes*
Bronchoaspiration	20	29	28	–	11
Urocolture	16	–	6	3	6
Emocolture	5	3	2	2	2
CVC colture	2	7	–	–	1
Other sites	5	–	–	1	1

Table 2 Antibiotic resistances

	Enterobacteriaceae	*Staphylococci*	*Pseudomonaceae*	*E. faecalis*
beta-lactams	25 (59.5%)	26 (63.4%)	15 (48.4%)	–
Fluoroquinolones	5 (11.9%)	–	16 (51.6%)	2 (33.3%)
Aminoglycosides	3 (7.1%)	–	16 (51.6%)	1 (16.7%)
Carbapenems	–	–	12 (38.7%)	–
Glycopeptides	–	–	–	1 (16.7%)

Conclusions: The main isolates were *Enterobacteriaceae*, *Staphylococci* and *Pseudomonaceae*, a result consistent with other studies; the main isolation sites were respiratory tract and urine. We found only one VRE (*E. faecalis*). The MRSA rate was lower than those of EPIC study (72.4%) and of an analogue study performed in Veneto (75%). *Pseudomonaceae* showed high rate of resistance to ceftazidime, fluoroquinolones and aminoglycosides (for *P. aeruginosa*, respectively: 42.8 vs. 27.7% in EPIC study, 46.4 vs. 26.3%, and 46.4 vs. 46%).

P1591 Two-year-point prevalence of infections in intensive care units in Piedmont (Italy)

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Objectives: To evaluate the prevalence of infections in intensive care units (ICU) and to compare the rates of two consecutive years.
Methods: There are 33 ICUs in Piedmont. In 2001, 18 nonspecialized ICUs took part into the study, while in 2002, 19 nonspecialized and 2 specialized (1 neurosurgery and 1 cardiovascular-surgery) were enrolled. The study population was defined as all the patients occupying a bed over a 24-h period on the study day. For all patients demographics, clinical status on admission (SAPS II), diagnostic and therapeutic interventions up to 1 week before the study day were recorded. The definitions of infections followed the 1998 CDC statements. Patient outcome was assessed up to 3 weeks after the study day.
Results: The study population (183 patients in 2001 vs. 201 in 2002) consisted of 254 male (126 in 2001 vs. 128 in 2002) and 130 female (57 in 2001 vs. 73 in 2002). The mean age was, respectively, 61 years in 2001 and 69 years in 2002. We found 109 infections in 2001 and 135 in 2002. The reported infections were as follows: ICU-acquired, 54 (49.5%) vs. 77 (57%); hospital-acquired, 29 (26.6%) vs. 34 (25.2%) and community-acquired, 26 (23.9%) vs. 24 (17.8%). The most frequently reported ICU-acquired infections involved the respiratory and the urinary tract (see table).

Type of infection	2001: *n* (%)	2002 : *n* (%)
Pneumonia + lower respiratory tract	31 (57.4)	33 (42.8)
Urinary tract	10 (18.5)	15 (19.5)
Bloodstream	6 (11.1)	14 (18.2)
Clinical sepsis	3 (5.6)	8 (10.4)
Wound	1 (1.8)	3 (3.9)
Gastrointestinal	2 (3.7)	2 (2.6)
Skin and soft tissue	1 (1.8)	1 (1.3)
Central nervous system	0	0

Conclusions: Most of the reported infections were ICU-acquired. About 30% of patients who had an ICU-acquired infection showed multiple infectious events. The infection rates in the 2 study years were similar to those of EPIC study, being lower than the Italian ones. No significant differences was observed when 2001 and 2002 were compared.

P1592 Prevalence of nosocomial infections in intensive care units in Turkey: a multicenter 1-day point prevalance study

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Objective: To determine the prevalence of intensive care unit (ICU)-acquired infections in Turkish ICUs. To identify associated risk factors, predominant infecting organisms, and mortality rates

Methods: A 1-day point prevalence study. A total of 56 ICU from 22 university or teaching hospitals were participated to the study. Coronary intensive care and pediatric intensive care units were excluded from the study. The study was carried out on September 19, 2001.
Results: A total of 236 completed case reports forms was accepted for analyzes. Eighty percent of these ICUs were situated from university hospitals and 20.0% in teaching community hospitals. 44.6% of the ICUs were surgical, 35.7% were mixed (medical and surgical) and 19.6% were medical. A total of 115 patients (48.7%) studied had one or more ICU related nosocomial infections on the study day. Pneumonia and lower respiratory tract infections (28.0%), laboratory confirmed blood stream infections (23.3%) and urinary tract infections (15.7%) were the most frequent types of ICU related infections. Of the nosocomial pneumonia, 81.9% was associated with mechanical ventilation, and 97.3% of urinary tract infections were associated with urinary catheters. Endotracheal tube, urinary catheter, multitrauma on admission, stress ulcer prophylaxis, nasogastric feeding, mechanically ventilation were the risk factors for ICU related nosocomial infections. The most frequently reported isolates for ICU related infections were as follows: *P. areuginosa* 20.8%, *S. aureus* 18.2%, *Acinetobacter* spp. 18.2% and *Klebsiella* spp. 16.1%. Of the 80.4% *Staphylococci* were resistant to methicillin. Of the *P. aeruginosa* isolates were resistant to amikacin (44.1%), ciprofloxacin (42.9%), imipenem (36.1%) and ceftazidime (44.7%). Resistance rates of *Acinetobacter* spp. for same antimicrobials were; 54.1, 60.5, 30.6, 74.3%, respectively. Of the patient 72.9% were receiving antimicrobials on the day of the study for treatment or prophylaxis. Most frequent administered antimicrobials were aminoglikozides percentage 37.2 (of the patients receiving antimicrobials), carbapenems 31.4%, glycopeptides 23.3%, cephalosporins 18.0% and 5.8% antifungals. At the end of 4 weeks follow up overall 70 (29.7%) patients died, 22 (9.3%) of them died from ICU infections.
Conclusion: This study showed that ICU related infections is common and often associated with resistant microorganisms

P1593 Antimicrobial resistance of microorganism isolated from nosocomial infections in intensive care units of a university hospital in Adana, Turkey

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Objectives: To describe the frequency and resistance rates of bacterial pathogens from patients admitted to four intensive care units at a university hospital.
Materials and methods: The isolates collected betweeen July 1999 and June 2001 included in the study were causes of nosocomial infection in intensive care units. The identification and susceptible to antibiotics has been performed in automated system Sceptor (Becton Dickinson) MIC/ID panels as described by the NCCLS.
Results: Among 3962 patients in the intensive care units, 492 infection episodes were diagnosed in 272 patients with nosocomial infection. Infection rate was 12.4%. Isolates from ICU patients were recovered from urinary tract infections (32%), primary blood stream infections (24%), nosocomial pneumonia (20%), surgical-site infections (13%) and other infections (11%). Pneumonia was the most common nosocomial infection (54%) in anesthesiology ICU. The pathogens causing these infections were 39% Gram-positive bacteria and 52% Gram-negative bacteria and 9% *Candida* species. *S. aureus* (18%) was the most frequently isolated bacteria, followed by *P. aeruginosa* (16%), *Acinetobacter* spp. (10%), Klebsiella (9%), *E. coli* (9%), CNS (10%), *Enterococcus* (9%). Resistance to oxacillin was observed for all of *S. aureus* isolates and 95% of the CNS isolates, all isolates were susceptible to vancomycin. 8% of *Enterococcus* isolates were resistant to vancomycin. Against the gram negative bacteria the carbapenems were most active with 61% of the isolates susceptible, followed by amikacin and ciprofloxacin. Five percent of *P. aeruginosa* and 15% of *Acinetobacter* spp. are found to be resistant to all antibiotics. The most active antimicrobial agents; against *Acinetobacter* spp. were (IMP) imipenem (73%), tobramycin (46%); against *Pseudomonas* spp. were Ticarcillin/clavulanate (56%), piperacillin (44%), IMP (33%); against *Klebsiella* spp. were IMP (92%), against *E. coli* were IMP (94%), AK (94%), CIP, ciprofloxacin (74%); against *Enterobacter* spp. were CIP (89%), IMP (78%).
Conclusions: The high rates of resistant pathogens responsible for nosocomial infection in ICUs suggest that infection preventive procedures should be implemented, antimicrobial agents must be properly used.

P1594 Methicillin-resistant *Staphylococcus aureus* nosocomial infection in the intensive care unit

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Introduction: The nosocomial infection (NI) due to methicillin-resistant *Staphylococcus aureus* (MRSA) is a growing problem in the world. In large tertiary hospitals outbreaks of MRSA are commonly encountered in critical care areas.
Objetives: We study the risk factors predisposing to NI by MRSA, with special emphasis on the prior antibiotic therapy to try to predict the development of this infection and to assess its clinical virulence.
Methods: Unmatched case-control study. The Hospital Central of Asturias is a teaching institution in north Spain. The hospital has a medical-surgical unit with an annual admittance rate of 400–500 patients. Our study was conducted at the Intensive Care Unit (ICU) for one year. All patients satisfying MRSA NI criteria were selected as cases. Controls were chosen among ICU-admitted patients with NI due to methicillin-sensitive *Staphylococcus aureus* (MSSA). The customary statistic study was completed with a stepwise logistic regression analysis.
Results: At the ICU setting, 47 patients with NI caused by MRSA were compared with the 39 controls who were available. Both groups had quite similar results with respect to the clinical and epidemiological characteristics. We failed to identify significant differences in severity between MRSA-infected and MSSA-infected patients (APACHE II index and SAPS index). Therapeutic interventions did not differ significantly between groups (TISS index). The most important differences between patients infected by MRSA and the controls were the length of their previous stay (median 19 vs. 4 days; $P < 0.001$), the frequency of prior infection (93.6 vs. 25.6%, $P < 0.001$) and the previous consumption of antibiotics (100 vs. 59%; $P < 0001$). The duration of antimicrobial therapy before the *Staphylococcus aureus* was isolated was significantly longer (17 vs. 6 days, $P < 0001$). A simple model allowed the prediction of the strain infecting with 86% success, considering the previous infection, the prior consumption of antibiotics and the age. Significant differences concerning mortality between the two groups were not found.
Conclusions: The previous administration of antibiotics was the most closely related factor to MRSA hospital-acquired infection both at ICU. In our study the clinical virulence of MRSA nosocomial infection was not greater than MSSA nosocomial infection, differences cannot be shown.

P1595 Source isolation in intensive care units does not reduce spread of MRSA beyond that achieved by universal precautions: a prospective two-center study

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Objectives: The incidence of methicillin resistant *Staphylococcus aureus* (MRSA) is increasing, particularly in ICU patients but many units lack sufficient single rooms and adopt universal 'standard precautions'. We conducted a two-center prospective study in two mixed ICUs to assess the benefits of source and cohort isolation over and above universal precautions.
Methods: Two general ICUs participated in the study over one year. All patients admitted for >48 h were studied. Patients were screened for MRSA colonization (nose/groin) on admission, and weekly thereafter. In the first and last three month periods (Phase I/III) MRSA positive patients were moved to single rooms or cohort bays but not moved in the middle six months (Phase II). Universal standard precautions for infection control were always used.
Results: The patient population was similar in each management phase. There was no significant difference between the two ICUs in compliance with

Table 1

	Phase I/III	Phase II	*P* value
Total number of patients staying >48 h	437	418	>0.1
No. (%) MRSA+on admission	68 (15.6%)	69 (16.5%)	>0.1
No. (%) becoming colonized with MRSA in ICU	50 (12%)	40 (11.3%)	>0.1
No. (%) infected with MRSA in ICU	15 (3.4%)	11 (2.7%)	>0.1
Outcome[1]	28 (6.6%)	27 (6.6%)	>0.1

[1]Total number of patients dying while active treatment for MRSA (defined as appropriate antibiotics within 48 h of death.

handwashing/disinfection regardless of patient dependency, or presence or absence of MRSA. The rate of acquisition of, or infection with, MRSA and mortality was similar whether patients were moved or not, and this was confirmed by multivariate analysis of risk.
Conclusion: Source or cohort isolation had no significant impact in decreasing MRSA spread in ICU patients when universal precautions were followed.

P1596 Nosocomial infections due to *Pseudomonas aeruginosa* in neonates

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Objectives: *P. aeruginosa* is one of the commonest cause of nosocomial infections in adults, and in children.
Methods: We investigated all neonatal infections caused by *P. aeruginosa* within 2 years among 246 newborns hospitalized in neonatal ICU. Univariate analysis to asses risk factors for neonatal infections caused by *P. aeruginosa* vs. neonatal infections caused by other organisms was performed.
Results: According to the analysis of risk factors for *Pseudomonas aeruginosa* infections, it was found, that ventilatory support (38.46 vs. 12.27%) and therapy with corticosteroides (15.38 vs. 3.18%) were significant predictors for *P. aeruginosa* infections. Positive blood cultures/catheter tips (38.46 vs. 9.55%) were more frequently observed in neonatal infections with *P. aeruginosa* than among neonatal infections without *P. aeruginosa*. Early marker for *Pseudomonas aeruginosa* infection was a higher procalcitonin level (PCT) (19.23 vs. 5.45%). Bacteremia (30.77 vs. 7.73%) and meningitis (7.69 vs. 0.45%) were the commonest sites of infection with *P. aeruginosa*, in comparison to the control group. Outcome was comparable in both groups.
Conclusion: Neonatal infections caused by *P. aeruginosa* are serious diseases causing significantly more meningitis and bacteremia than other organisms. Mortality rate 3.85% was lower than in adults (25%), probably due to early diagnosis and appropriate therapy with meropenem or ceftazidime, in a combination with an aminoglycoside, used in our neonatal referral center.

P1597 Molecular epidemiology of multiresistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates at intensive care units: the importance of surveillance cultures

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Objective: To describe the clonal relationship of multiresistant *P. aeruginosa* and *A. baumannii* which were recovered from clinical specimens of intensive care unit (ICU) patients and surveillance cultures.
Methods: Between May 2001 and February 2002 a prospective study on the epidemiology of multiresistant Gram negative bacteria (GNB) was conducted in the three ICUs (Anaesthesiology and Reamination, Internal Medicine, Neurosurgery) of Dokuz Eylul University hospital located in Izmir, Turkey. Surveillance cultures for GNB were obtained weekly from the patients, inanimate surfaces, and the hands of personnel. Clinical isolates were also collected during the study period. Isolates were analyzed for clonality by ERIC PCR.
Results: A total of 40 *P. aeruginosa* isolates (28 clinical, and 12 surveillance culture isolates) and 80 *A. baumannii* isolates (10 clinical, and 70 surveillance culture isolates) were taken into the study. It was observed that two clones each of *P. aeruginosa* and *A. baumannii* had been disseminated at the ICUs of the hospital. Organisms which shared the same ERIC PCR pattern with the clinical isolates were recovered from the hospital environment and the hands of healthcare personnel. Although a distinction could not be made among *P. aeruginosa* isolates according to the resistance profile; *A. baumannii* clusters could be differentiated by imipenem susceptibility.
Conclusion: Epidemiological links between hand culture, environmental and clinical isolates suggested cross transmission of multiresistant *P. aeruginosa* and A.baumannii in the ICUs. The importance of surveillance cultures were shown as colonization preceded infection in several patients. In addition, the visits and surveillance culture results increased the compliance of healthcare and other personnel to infection control measures.

P1598 Study colonization of nosocomial pathogens in tracheal tubes of hospitalized patients in intensive care units of Iranian hospital

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Objective: To determine colonization rate of nosocomial pathogens in tracheal tubes of Patients hospitalized in intensive care unit of Milad Hospital and performance of susceptibility testing for high frequency isolates.
Methods: Between July 2001 to November 2002, 249 endotracheal aspirates specimen from patients hospitalized in ICU and NICU of Milad hospital were sent to microbiology laboratory. All specimens were processed according standard microbiologic methods and all isolated microorganisms identified by routine phenotyping methods. Antimicrobial susceptibility testing of all isolates were determined with disk diffusion methods according NCCLS guideline M100-S12.
Results: Of 249 endotracheal aspirates 222 (89%) specimen yielded growth of microorganisms. In total 335 microorganisms isolated. Of 46 (18.4%) specimens more than one microorganisms isolated. Endotrachael aspirates cultures of this patients showed decreasing frequency of *Klebsiella pneumonia* 67 (20%), *Pseudomonas aeurginosa* 62 strains (18.5%) *Acinetobacter* spp. 60 (18%) strains, *Staphylococcus aureus* 51 (15.2%) strains, *Seratia marcescens* 32 (9.5%) strains, *Escherchia coli* 22 (6.6%) strains. *Enterobacter* spp. 10 *Candida albicans* 5 isolates (1.5%) and miscellaneous 26 isolates (7.7%). More than 90% of isolates of *S. aureus* were resistant to oxacillin. All isolates of *S. aureus* were resistant to vancomycin. Resitance rate of *K. pneumonia* to ciprofloxacin was 24% and all isolates were resistant to ampicillin. More than 90% of isolates were resistant to other commonly used antibiotics. All isolates of *Acinetobacter* were multidrug resistant. Susceptibility of *P. aeruginosa* to ciprofloxacin and amikacin was 66 and 50%, respectively.
Conlcusion: This study revealed a high frequency colonization of drug resistant nosocomial pathogens in tracheal tube of ICU hospitalized patients. These organisms are major causative agents of nosocomial pneumonia.

P1599 Bacterial colonization of patients in intensive care unit

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Objective: It is claimed that the origin of resistant microorganisms in the intensive care unit may be the admission of patients with bacterial colonization or infection with these resistant species from other units. We aimed to evaluate the bacterial colonization of intensive care unit patients at their admission.
Methods: Nose and throat specimens of 80 patients were taken within 24 h of their admission to intensive care unit between April 2002 and January 2003. Both Gram-negative bacilli and methicillin resistant *Staphylococcus aureus* (MRSA) were accepted as bacterial colonization. The methicillin sensitive *Staphylococus aureus* (MSSA) species which is normal for flora were accepted as bacterial colonization if they colonized pure and/or intensively.
Results: Bacterial colonization were detected 33 of 80 (41.2%) patients from their nose and throat specimens. A total 37 different colonization agent were determined.

Origin of patients	No:	Colonized patients	MRSA	Ent	Ps, As	Candida	Total
Other units	34	21 (61.8%)	8	11	7	1	27
Other hospital	1	1 (100%)		1			1
Community	45	11 (24.4%)	3	5		1	9
Total	80	33 (41.2%)					37

MRSA: Methicillin resistant *Staphylococcus aureus*, Ent: *Enterobactericeae*, Ps: *Pseudomonas* spp, As: *Acinetobacter* spp

Conclusion: Primary nose and throat specimens of patients on their admission to intensive care unit may be useful in predicting resistant microorganisms and isolation.

P1600 Dynamics of bacterial fecal colonization of patients at a horacic intensive care unit

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Objectives: The aim of this study was to examine the dynamics of fecal colonization of patients admitted to a thoracic ICU (TICU) at the University Hospital of Linköping, Sweden
Methods: Patients admitted to the TICU between November 2001 through January 2002, that had a length of stay more than 5 days were included and age, gender, cause of admission, length of stay, antibiotics administered, cultures (species, source, antibiotic susceptibility) taken during the stay on clinical indications were recorded. Rectal swabbing was performed on all patients during their stay at the TICU. Two selective chromogenic UTI medium (Oxoid) were used: one for enterococci with addition of 10 mg/L cephalexin, 75 mg/L aztreonam, 15 mg/L amphotericin B and one for Gram-negative bacteria with addition 32 mg/L vancomycin and 15 mg/L amphotericin B. Susceptibility testing was performed by disc diffusion and *E*-test (AB Biodisk)
Results: Eleven patients were included, the majority for postoperative care after coronary by pass surgery and/or cardiac vault surgery. Length of stay varied between 8 and 47 days (mean 23 days). Each patient received between 2 and 10 different antibiotics during their stay. Rectal swabbing was performed on each patient between 1 and 8 times. In seven patients rectal swabbing was performed $\geq$2 times, among these patients an initially mixed Gram-positive and -negative flora was replaced with only Gram-positive bacteria in five patients. Among patients where the same species was found in more than one culture, a development of resistance was seen. Eight of 11 patients (73%) were colonized with *Enterococci*. This was the most commonly found bacteria followed by *E. coli* (45%) and coagulase negative *Staphylococci* (CoNS) (45%). In cultures taken on clinical indications CoNS was the most common bacterium. Pathogens found both as faecal colonization and in clinical cultures from the same patient were: CoNS (4 patients: 3 central venous catheter, 1 introducer), *P. mirabilis* (2 patients: 1 introducer, 2 respiratory tract), *E. faecalis* (1 patient: central venous catheter), *S. maltophilia* (1 patient: respiratory tract), *E. cloacae* (1 patient: abdominal fluid, respiratory tract), *K. pneumoniae* (1 patient: wound).
Conclusion: In several patients the Gram-negative bacteria of the fecal flora disappeared during long ICU stay and only Gram-positive bacteria were detected, and among these a development of resistance was seen.

P1601 Enviromental, equipment and health care workers contamination during multidrug-resistant *Acinetobacter* outbreak in intensive care units of an Iranian hospital

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Obejctive: The aim of this study was to determine contamination of environment,equipment, and skin of personnel's hands in intensive care units (ICU) by *Acinetobacter baumannii* in Milad Hospital of Tehran.
Methods: Behind outbreak of infections caused by multidrug-resistant strains of *Acinetobacter baumannii* in ICU of Milad Hospital we decided for sampling to detect environmental contamination by this organism. In total 112 sample were taken from various sections and equipment and personnel's skin hand. All specimens processed according routine microbiologic methods. Susceptibility testing performed according NCCLS M100-S12 guideline.
Results: Of 121 environmental samples obtained with swab and skin samples 29 (30%) were positive for *Acinetobacter baumannii*. The most equipment contaminated with *A. baumannii* included: ventilator tubes personnel skin hands, tap water bedsides ambuobags tracheal tubes desks and computers. Of 27 personnel we isolated *A. baumannii* from hand of 5 (18.5%) staffs. All isolates were resistant to commonly used antibiotics such as amikacin, cefepime ceftazidim and cefotaxim. Both environmental and personnel isolates had the same phenotyping character, but genotyping could not be performed.
Conclusion: Environmental contamination may be an important reservoir in outbreak of infections caused by *A. baumannii* which in this cases implementation of infection control measures recommended for controlling infections

P1602 Hospital-acquired bacteremia due to *Acinetobacter baumannii* in critically ill patients

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Objectives: *Acinetobacter baumannii* is a common cause of bacteremia in ICUs, frequently associated with pneumonia and use of catheters. The aims of this study were to know the epidemiology, clinical features and predictors of mortality of nosocomial bacteremia due to *Acinetobacter baumannii* in our setting.
Material and methods: During a five and a half-year period (1996–2001) we have evaluated all clinical significant hospital-acquired ICU-bacteremias of a teaching hospital (12 beds) and specially those due to *Acinetobacter baumannii*. Epidemiologic clinical and microbiologic features were recorded from clinical charts. Multivariate analysis was performed to determine the predictors of mortality by using SPSS package (9.0)
Results: Fifty-three (30%) from 174 hospital-acquired bacteremias in critically ill patients were due to *Acinetobacter baumannii*. The mean age of patients was 59.5 ± 18.3 years, and the distribution by sex was 75% men and 25% women. A rapidly or ultimately fatal underlying disease was present in 38% of cases. Ninety-two percent of patients had indwelling vascular catheters and 90% were mechanically ventilated. Septic shock was present in 17% of patients. The origin of infection was respiratory in 43%, catheter 17%, abdominal 2%, meningitis 2% and unknown 36%. Ninety percent received prior antimicrobial therapy, 89% of isolates were multidrug resistant and inappropriate empiric antimicrobial treatment was given in 36% of cases. In 25% of cases the bacteremia was polymicrobial. The crude mortality rate was 53% and the related mortality to bacteremia 23%. The age of the patients ($P = 0.026$) and the presence of inappropriate empirical antimicrobial treatment ($P = 0.023$) were identified as independent predictors of mortality using multivariate analysis.
Conclusions: Multiresistant *Acinetobacter baumannii* was a common cause of lCU-acquired bacteremia (30%), with high mortality rate. In this study we identified two independent predictors of mortality: age of patients and inappropriate empirical antimicrobial treatment.

P1603 Ventilator-associated pneumonia in patients with trauma: risk factors concerning mortality

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Objective: The aim of this study was to evaluate the risk factors of death in ventilator-associated pneumonia (VAP) in patients with trauma.
Methods: One hundred consecutive adult patients with multiple trauma who received more than 24 h ventilatory support in intensive care unit (ICU) of municipal emergency hospital were evaluated prospectively. Two patients were excluded from the analysis because of early death. VAP was defined according to presence of CDC criteria. Causative microorganisms were detected by quantitative culture of mini-BAL or blind protected specimen brush and their susceptibility to antibiotics was performed by disk diffusion method. Age, sex, level of consciousness (Glasgow Coma Score), surgical procedures, nutritional status, antibiotic usage, APACHE II, approach to gastrointestinal protection, period of ventilation were evaluated as the possible risk factors concerning mortality in VAP. Patients with COPD were not included in the study. Plural factor of correlation and Student's *t*-test were used for statistical evaluation.
Results: VAP was diagnosed in 68 of 98 patients (69.4%). Overall mortality was 47.1% (32/68) and in 26 of 32 (81.3%) died patients the pneumonia was the direct reason of death. Sex and age of patients did not significantly influence the outcome of VAP. Risk factors leading to death in VAP patients with trauma were duration of mechanical ventilation more than 48 h (plural factor of correlation 0.51), APACHE II more than 20 points (0.48), proven aspiration (0.78), inadequate initial antibacterial therapy (0.56). The presence

of *P. aeruginosa* as etiologic agent of VAP also was associated with mortality (63% in died patients vs. 25% in cured patients, $P < 0.005$).
Conclusion: VAP is the most important complication of mechanical ventilation in ICU. Duration of mechanical ventilation more than 48 h, APACHE II more than 20 points, aspiration, inadequate initial antibacterial therapy and *P. aeruginosa* are the independent risk factors leading to death in VAP patients with trauma.

P1604 Postoperative pneumonia

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Objectives: An assessment of empiric therapy of pneumonia in surgical patients in relation to clinical disease, severity of infection and etiology.
Methods: The study was open and prospective. Patients with diagnosis of pneumonia, treated in surgical department or ICU in 1996–2001 were included. Diagnosis was based on clinical signs and symptoms, chest radiograms and positive bronchial aspirate cultures. Antibiotics were usually started before bacteriologic diagnosis was available.
Results: A total of 140 patients with pneumonia (developed most frequently postabdominal surgery) were included into the study. Patients were divided in two groups in relation to clinical disease: early postoperative pneumonia (EP) in 74 patients, developing in the first week following surgery and often with moderate clinical course (72%) and late septic complication in 66 patients with acute respiratory failure necessitating mechanical ventilation (VAP), usually in 2nd–4th week. In the VAP group septic syndrome was diagnosed in 89% and septic shock in 11% of patients. Incidence of pneumonia was higher after emergency surgery and reoperation (58 and 68%). In EP group concomitant abdominal sepsis was diagnosed in 34% and in VAP group in 62% of patients. Among isolated pathogens, Gram-negatives prevailed (79%): mainly *K. pneumoniae*, *E. coli* and *E. cloacae* in EP group and *P. aeruginosa*, *Acinetobacter* spp., *E. cloacae*, *S. marcescens* in mixed infections with *S. aureus* or *Enterococci* (36%) in VAP group. Empiric therapy in EP group included ceftriaxone, ciprofloxacin and cefepime in the last 2 years. In VAP group, most frequently imipenem/cilastatin in monotherapy or with amiglycoside or cefepime with metronidazole in patients with abdominal sepsis were used. Empiric therapy had to be modified in 36% of patients in EP group because of selection of resistance to ceftriaxone among enteric rods. In VAP group modification of therapy (69%) was related mainly to superinfections with MRSA, *Enterococci*, *C. difficile*, *S. maltophilia* and *Candida* species. Mortality rate was closely related to clinical diseases, and in VAP group was 45%.
Conclusions: In severe pneumonia with respiratory failure imipenem and cefepime are the most rational and useful drugs for empiric therapy. Older cefalosporins, as ceftriaxone, are still of substantial importance in moderate clinical disease.

P1605 Incidence of sepsis and septic shock in various hospital wards (results of the epidemiological survey of the multicenter study 'IRIS')

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Introduction: Although ACCP Consensus Conference criteria of sepsis and related conditions were introduced in 1992, no epidemiologic studies of sepsis were conducted in Russia that used uniform criteria to evaluate epidemiology of sepsis and septic shock.
Objective: To determine incidence of sepsis and septic shock in various hospital wards in Russia.
Methods: Case histories (CS) of hospitalized patients (adults and pediatrics) from various hospital wards in eight cities throughout Russia were evaluated to determine incidence of sepsis and septic shock. ACCP Consensus Conference, 1992 definitions of sepsis and septic shock were applied. CS were taken for 3-month period from October 1 to December 1, 2001.
Results: Overall, 7445 CS of hospitalized patients were analyzed. Among them, 2309 CS were from general surgery wards, 1948 – from infectious surgery wards, and 1202 – from therapeutic wards. From adult ICU 1474 CS and from pediatric intensive care units, 512 CS were evaluated. The percentage of patients with infections was 94% in infectious surgery wards, 48% in adult ICUs, 42% in pediatric ICUs, 22% in therapeutic wards and 20% in general surgery wards. Sepsis in above mentioned patients was revealed in 51% of cases in adult ICUs, in 16% of cases in infectious surgery wards, 13% in therapeutic wards and 11% in general surgery wards. In pediatric ICUs symptoms relevant to sepsis were discovered in 22% of patients with infections. Septic shock has occurred in 41% of patients with sepsis in adult ICUs, in 14% of patients with sepsis in pediatric ICUs and in 10% of patients with sepsis in infectious surgery units.
Conclusion: The results of the study show that sepsis is a real problem in Russia. There is a major divergence between the incidence of sepsis and septic shock evaluated with application of international criteria and official data. Further educational efforts and implementation of international criteria of sepsis are requested.

P1606 Are infections with resistant pathogens worsening the outcome of surgical ICU patients?

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Background and goal of study: Our hypothesis was that the outcome of ICU patients in whom an infection with multiresistant microorganisms was proved could be worse than in patients infected with susceptible strains.
Materials and methods: Data were retrospectively collected for patients admitted between 1997 and 2002 in a surgical ICU. The following parameters were compared: SAPS II score, length of stay (LOS) in the ICU, LOS in ICU before the infection was proved, mechanical ventilation (MV) free-days, hemodialysis (HD) free-days, the use of antibiotics (AB) before infection was proved, ICU and in-hospital mortality. CDC criteria were used to define infection. Data were compared using analysis of variance (ANOVA) method.
Results: We included 515 patients of whom 136 patients had an infection with multiresistant strains. We found multiresistant species of *Pseudomonas aeruginosa* in 45 cases, *Enterobacter* spp. in 38 cases, *Acinetobacter baumanii* in 28 cases, *Enterococcus* spp. in 15 cases and MRSE in 10 cases. There were no significant differences between SAPS II score, MV and HD free days, ICU and in hospital mortality. LOS before infection was observed and the total ICU LOS were longer in patients infected with multiresistant strains. Also the previous use of antibiotics was significantly higher in this group. Results are presented in the table.

	Resistant strains	Susceptible strains	*P* value
SAPS II	30 ± 2	29 ± 2	NS
LOS	18 ± 6	12 ± 3	0.03
LOS before infection	8 ± 3	5 ± 3	0.03
MV free days	8 ± 2	7 ± 3	NS
HD free days	9 ± 1	8 ± 2	NS
ICU mortality	40/136 (29%)	108/379 (26%)	NS
In hospital mortality	8/136 (6%)	16/379 (4%)	NS
Previous AB	80/136 (59%)	118/379 (31%)	0.02

Conclusions: Our results show that there is no difference between the outcome of ICU patients infected with multiresistant microorganisms and patients infected with sensible strains. Previous use of antibiotics and longer ICU stay were associated with infection with resistant strains.

P1607 Epidemiologic investigation of an outbreak due to imipenem-resistant *Klebsiella pneumoniae* in the ICU

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Background: During a period of approximately 3 months (17/9–12/12/02) an outbreak of imipenem-resistant *K. pneumoniae* was detected in our hospital which included six patients in the ICU. Soon after the first two cases were identified, an epidemiologic investigation was begun in the ICU including patients, personnel and the environment.
Methods: *K. pneumoniae* was identified using the Vitek System. Disk diffusion testing was performed by the Kirby-Bauer method according to NCCLS guidelines. Imipenem MICs and metallo-beta-lactamases production were determined by the diffusion based *E*-test MBL (AB Biodisk, Solna, Sweden). Positive for MBL production isolates of *K. pneumoniae* were then screened by PCR assay for genes of the blaVIM family. Transferability of plasmids carrying the *blaVIM* genes were investigated by conjugation experiments. Molecular typing of VIM + isolates was performed by ERICII-PCR assay. Hand cultures were performed on ICU personnel. Environmental samples were

collected from sinks, disinfectants, bedding, stethoscopes, telephones and mechanical ventilator system equipment.
Results: Fourteen imipenem-resistant *K. pneumoniae* strains were isolated from different clinical samples of six patients. The epidemiologic investigation revealed that the only environmental source was the monitor keyboard of the mechanical ventilator system of one of the patients and none of the stuff members harbored imipenem-resistant *K. pneumoniae*. All clinical isolates and the environmental isolate exhibited the same antibiotic resistant profile and were tested resistant to all major classes of antibiotics and sensitive only to colistin. Moreover, all isolates produced blaVIM metallo-beta-lactamases and were genetically related as shown by ERICII-PCR assay. Plasmids carrying *blaVIM* genes were easily transferred to *E. coli* recipients.
Conclusions: These data suggest the hypothesis of a clonal origin of the strain and a cross infection from patient to patient through the contaminated surface via the hands of the personnel. The emergence of *K. pneumoniae* resistant to imipenem is a new threat particularly in the ICU environment. Clinicians and laboratory personnel must be aware and cooperate for early identification of the microorganism and prevention of its spread.

P1608 Hyperproduction of AmpC among *Enterobacter* spp. isolates from intensive care patients in a Spanish hospital

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Background: Hyperproduction of AmpC in *Enterobacter* spp. makes these bacteria resistant to most beta-lactam antibiotics. Derepressed mutants can be selected from inducible populations during antimicrobial therapy. Once selected, mutants are stable and can persist in the hospital microflora. In Hospital Universitari de Bellvitge (HUB), the rate of derepressed *Enterobacter* spp. (DEb) isolated from clinical samples among ICU patients was 48.6% (17 out of 35 isolates) during the first 6 months in 2002. A 5-month study (July–November, 2002) was carried out to determine the epidemiology of Deb in our ICU.
Objectives: (i) To determine the number of patients colonized by DEb in the ICUs in a 5-month-period; (ii) to characterize the number of infections cause by DEb; and (iii) to analyze the clones detected.
Methods: Colonization was detected from rectal and axillar swabs taken weekly from ICU patients until ICU discharge. Specimens were processed in McConkey plates supplemented with cefotaxime (2 g/L) and ceftazidime (4 g/L). Antibiotic susceptibility and identification of isolates were performed by MicroScan. Double disk-diffusion tests were used to detect extended-spectrum beta-lactamase production (ESBL). Genotyping was done by pulsed-field gel electrophoresis.
Results: Colonization studies were performed on 190 patients, 46 of them were colonized by DEb (24%) (19 *E. cloacae* and 27 *E. aerogenes*). Among colonized patients, 20 (43.5%) had positive clinical samples for DEb (9 *E. cloacae* and 11 *E. aerogenes*). Isolates in 45 patients had a resistance pattern compatible with AmpC hyperproduction. In one patient, colonization by *E. aerogenes* ESBL-producer was detected. Isolates from 43 patients were available for genotyping, and 41 restriction patterns were found. In nine patients paired strains (clinical and colonizing isolates) were typed, and were identical in all but three patients.
Conclusion: The DEb colonization rate detected among ICU patients in HUB was high (24%). In contrast, 10.5% of the patients studied had clinical samples with DEb and 5.3% had well-documented infections. With the exception of two cases, no cross transmission was found. Thus, most DEb might have arisen among patient's digestive microflora under selective antibiotic pressure.

Gastro-intestinal infections

P1609 Enteropathogenic bacteria in adult patients with diarrhea: frequency of isolation and susceptibility to commonly used antimicrobial agents (a 3-year study)

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Objectives: To study the bacterial etiology of the inflammatory diarrhea, the frequency of isolation of various enteric pathogens, the predominant species and serotypes and their susceptibility to commonly used antimicrobial agents.
Materials and methods: During the last 3 years, 1230 patients with diarrhea were studied. The laboratory examination included: (a) direct microscopy of stool wet preparation for leukocytes and erythrocytes; and (b) stool culture for enteric pathogens *Salmonella, Campylobacter, Shigella, Yersinia enterocolitica* and *Aeromonas* with the standard laboratory techniques. The *Shigella* subgroups and the *Y. enterocolitica* serogroups were identified by slide agglutination test with antisera, while the serotypes of *Salmonella* were determined in the reference center. The antimicrobial susceptibility testing was carried out by disk diffusion test and the MICs were determined by *E*-test and by the automated system Vitek 2.
Results: Enteropathogenic bacteria were isolated in 174 out of the 1230 (14.15%) stool specimens examined by culture (one specimen/patient). *Salmonella* with predominant serotype *S. enteritidis* was the commonest enteric pathogen isolated (69.55%), followed by *Campylobacter* (23.56%), while *Shigella* (predominant subgroup *S. flexneri*), *Y. enterocolitica* (serogroup 0 : 3) and *A. hydrophila* were isolated in low proportions (4.02%, 1.72% and 1.15%, respectively). Leukocytes were detected in 156 (89.65%) out of the 174 cases of inflammatory bacterial enteritis. The antimicrobial susceptibility testing showed high resistance rate of *Salmonella* and *Shigella* to doxycycline (43.8% and 57.14%), lower to ampicillin (9.91% and 28.6%) and very low to trimethoprim–sulfamethoxazole (1.65% and 14.3%), respectively. Ten of the 41 (24.4%) *Campylobacter* isolates (almost exclusive species of *C. jejuni*) were found resistant to ciprofloxacin (MIC > 32 μg/mL), while all the isolated strains were susceptible to erythromycin.
Conclusions: *Salmonella*, followed by *Campylobacter* are the most common causative agents of bacterial diarrhea in our country. Despite the very low resistance rate of *Salmonella* and *Shigella* to trimethoprim–sulfamethoxazole, the fluoroquinolones remain the drug of choice for the treatment of these enteric pathogens, while erythromycin is the drug of choice for the treatment of diarrhea caused by *Campylobacter* (when needed), because of the high resistance rate to ciprofloxacin.

P1611 Isolation of *Arcobacter skirrowii* in a patient with chronic diarrhea

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Objectives: The genus *Arcobacter* includes four species. *A. butzleri* and *A. cryaerophilus* have been associated with human disease. *A. nitrofigilis* has been recovered from the roots of a salt marsh plant. The fourth species *A. skirrowii* was found in gastroenteritis and abortion in farm animals. Until now, no association of this species with human infection has been described. We report here a case of diarrhea in an elderly patient from whom *A. skirrowii* was isolated.
Methods: A 73-year-old man was admitted to the hospital after 2 months of persisting diarrhea. The diarrhea had not responded to dietary measures and treatment with nifuroxazide. The patient lost weight, was anorexic and became astenic. He was treated for progressive coughing with levofloxacine for several days just before hospitalization. A colonscopy showed diverticulosis but no malignancies. His symptoms resolved during his fortnight stay in the hospital. Stool specimens were cultured for conventional enteric pathogens. In addition to a selective *Campylobacter*-medium incubated at 42°C, a filtered fecal suspension, was inoculated on Columbia blood agar and incubated at 37°C.
Results: The conventional cultures for enteric pathogens and *Campylobacter* were negative. The Columbia agar plate showed very slow growing, oxidase positive colonies of Gram-negative curved rods. The strain was able to grow at 15°C, which is a distinctive feature to differentiate *Arcobacter* species from

Campylobacter species. A multiplex PCR, developed for the simultaneous identification of *A. butzleri, A. cryaerophilus* and *A. skirrowii*, identified the organism as *A. skirrowii*.
Conclusions: We believe this to be the first isolation of *A. skirrowii* from a human stool sample. It is not clear whether this strain was etiologically associated with the patient's diarrhea. Because of their fastidious growth, especially pronounced in *A. skirrowii*, it is not easy to get an insight into the occurrence of *Arcobacter* in human disease. However 20 years experience with *Campylobacter* filtration techniques gives us reason to believe that the role of *A. skirrowii* in human disease is limited.

P1612 *Cryptosporidium gastroenteritis*: a retrospective study of 113 patients

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Objectives: Prevalence rates of *Cryptosporidium* among children with diarrhea are between 3 and 4% in industrialized countries, excluding outbreaks which mainly occur in day-care centers. The objective of this study was to assess epidemiological features of infection with this parasite in a children population.
Methods: Data was drawn from the records of 113 patients admitted between 1984 and 2000. All patients were < 14 years of age and identification of the oocysts was confirmed in their stool.
Results: A total of 113 patients (66 males); 47.7% were <24 month, 31.8% were 24 month $< n <$ 5 years old. There was a peak incidence in 1989 (13 cases) and 1998 (15 cases). Seasonal distribution revealed a rise in cases in months May through August. On admission, 50% had signs of mild dehydration and only three severe. Fever was present in 63% with an average duration of 3.3 days. Vomiting was a symptom in 88.5%. Fifteen percent had severe abdominal pain. Diarrhea was watery in 71.7%; 61% had mucus and only 9.7% had blood in their stool. Convulsions occurred in two patients. Intravenous fluid administration was necessary in 54.8% for a median of 48 h. Nine patients received iv antibiotics that were discontinued when diagnosis was confirmed. Laboratory findings revealed 11 patients with anemia (Hgb < 10 g/dL). The WBC had a median value of 8200 (SD = ±3181.8) and there was no marked granulocytosis. Leukopenia (WBC < 5000) was present in five patients. The average duration of hospitalization was 3.9 days. The course of disease was uncomplicated in all cases and no deaths occurred.
Conclusions: *Cryptosporidium gatsroenteritis* is a self-limited disease and no antimicrobial therapy is required. It mainly concerns children <2 years of age and the prevalence remains low.

P1613 Four cases of rhabdomyolysis related to *Salmonella enteritidis* gastroenteritis

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Objectives: Rhabdomyolysis is a disorder characterized by elevated serum concentrations of CK due to skeletal muscles injury: it is often a complication of a broad spectrum of diseases related to infectious and noninfectious causes. It is rarely associated with gastroenteritis: respiratory and urinary infections and catheter-related sepsis are the main causes of infectious RM and Gram-negative organisms are more representative than positive ones. We describe four rhabdomyolysis cases during GE caused by *S. enteritidis* in a group of 14 policemen, observed in the Infectious Diseases Department, S. Anna Hospital, Como.
Methods: All the patients came to our observation for an acute GE. We performed clinical examination, stool culture, parasitic fecal analysis, blood cultures (in case of body temperature >37.5°C) and routine laboratory analysis, including CPK. In patients showing CPK increase: ECG, mass-CPK, mioglobin and troponin-I were performed too. Rhabdomyolysis diagnosis was stated with GABOW criteria.
Results: The results are shown in Table 1.

Table 1

	Age (years)	Sex	CPK (mU/mL)	Mass-CPK (mg/mL)	Mioglobin (mg/mL)	Troponin (mg/mL)
Case 1	27	M	38860	1.3	1083	0.01
Case 2	56	M	2227	1.3	255	0.01
Case 3	25	M	1018	1.3	350	0.01
Case 4	31	M	8350	1.3	820	0.01

The reported cases show a relevant increase in CPK values, without any increase of acute myocardial infarction markers. Creatinine values were normal too.
Conclusions: Early recognition of rhabdomyolysis important to avoid severe and potentially lethal complications, like acute renal failure. In our study, we consider relevant the percentage of subjects affected by RM: 4/14 (28.4%), all related to acute gastroenteritis caused by *S. enteritidis*.

Experimental bacterial infections

P1614 Role of lipo-oligosaccharide on the attachment of *Moraxella catarrhalis* to human pharyngeal epithelial cells

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Objective: Bacterial attachment to host cell is the initial step in the pathogenesis of infections. It has been shown that attachment of *Moraxella catarrhalis* to human pharyngeal epithelial cell (HPEC) is basis of colonization and subsequent infections. In *M. catarrhalis* fimbriae act as an adhesin, however, there are several other surface structures, which may act as an adhesin. In several bacterial strains, it has been shown that multiple adhesin–receptor interaction occur for firm binding of bacteria with host cells. As a major constituent of surface structures it has been shown that lipopolysaccharide/lipo-oligosaccharide (LPS/LOS) of several bacteria acts as an adhesin, which mediate the attachment to host cells. Therefore, this study was done to find out the role of LOS on the attachment of *M. catarrhalis* to HPEC.
Methods: Strain 2951, a *M. catarrhalis* strain and its galE mutant which lack the (Galalpha1–4Galbeta1–4Glc) P(k) epitope of LOS, were used in this study (kindly provided by Prof. Michael Apicella, University of Iowa, USA). Assay was done to find out the attachment ability of both strains. Thin layer chromatography (TLC) was done to compare the binding ability between the wild type and mutant with different glycolipids. For the determination of surface charge, *M. catarrhalis* was treated with cationized ferritin particles and observed under a transmission electron microscopy (TEM). Surface potential was detected in both strains by atomic force microscope (AFM) equipped with surface potential spectroscope.
Results: HPEC taken from 10 healthy normal adults shows that the attachment of mutant strain (25.2 ± 9.3 bacteria/cell) was significantly ($P < 0.001$) less than the wild strain (38.3 ± 15.4). In both strains, a positive reaction was obtained in TLC with Gg4Cer and Gg3Cer, but no reactivity was observed with ganglioside GM1, GM2, GD1a, GD2, GT1b and GQ1b. Both AFM and TEM showed that the wild type strain is negatively charged. While the mutant strain has less negatively charged areas.
Conclusion: This study suggests that (Galalpha1–4Galbeta1–4Glc) P(k) epitope of LOS may be one of the factors for the negative surface charge of *M. catarrhalis* and intact LOS is necessary for the attachment of *M. catarrhalis* with HPEC. The binding of both the wild and the mutant strains with Gg4Cer and Gg3Cer indicate that other receptor on the HPEC might be involved in the attachment of *M. catarrhalis*.

P1615 Increased mortality and spatial memory deficits in TNF-alpha deficient mice after experimental pneumococcal meningitis

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Objectives: Tumor necrosis factor alpha (TNF) is critically involved in inflammation and cellular immune response during bacterial infections.

TNF may participate in hippocampal neuronal injury in meningitis. In a mouse model of pneumococcal meningitis, the influence of TNF-deficiency on mortality and on spatial memory performance was investigated.
Methods: 57 TNF-deficient mice (males, 2–3 months) and 55 sex- and age-matched controls (C57Bl6) were trained to find a hidden under-water platform within less than 90 s (18 trials over 3 days). Swim tracks and the latency to escape from the water were recorded by a video camera. Thereafter, mice were infected by injection of 4 log cfu *Streptococcus pneumoniae* into the right forebrain. 30 h later therapy was initiated with ceftriaxone (100 mg/kg twice daily for 5 days). Motor performance was measured by the tight rope test. Beginning 7 days after infection, water maze was performed (daily in the first week, then three times per week), and mice were killed 6 weeks after infection.
Results: During swim training in the first 3 days no differences were seen in the water maze between TNF-deficient mice and controls. After infection, tight rope test showed severe impairment during the acute phase of meningitis. A total of 35 (61%) TNF-deficient mice and 22 (40%) controls died within 6 days (Fisher's exact test: $P = 0.04$). All other animals fully recovered from the infection. TNF-deficient mice surviving pneumococcal meningitis took substantially longer to reach the hidden platform than controls ($P = 0.02$) and the distance of swim tracks was significantly longer ($P = 0.02$). The swim speed in both groups was similar ($P = 0.59$).
Conclusions: In pneumococcal meningitis, TNF deficiency caused increased mortality and deficits in spatial memory, i.e. the increased vulnerability of TNF-deficient animals to infection outweighs possible detrimental effects of TNF release within the CNS.

P1616 Meropenem acts synergistically with levofloxacin against penicillin-resistant pneumococci in experimental meningitis and prevents levofloxacin-induced resistance in vitro

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Objectives: The bactericidal activity of meropenem (MEM) combined with levofloxacin (L) in experimental meningitis and its effect on quinolone-induced resistance in vitro were investigated.
Methods: Two penicillin-resistant (penR) strains were used with identical MICs (mg/L): L:1; MEM:0.5; penG:4. Both strains were used in time-killing experiments in vitro over 8 h and in checkerboards. WB4 was used in the rabbit meningitis model.
Results: The bactericidal activity of the different treatment groups are expressed as killing rates/8 h (delta log 10 cfu/mL 8 h). Untreated controls (N: 5): $+0.28 \pm 0.11$; MEM (N: 8): -3.80 ± 0.90; L(N: 8): -3.40 ± 0.66; MEM + L(8): -6.0 ± 0.9. All CSF samples treated with the combination were sterile after eight synergy between MEM and L was also demonstrated in vitro in time-killing assays over 8 h and with the checkerboard method. Sequential exposure of the two penR strains (WB4and KR4) to L led to a 64× increase of the MIC after 10 cycles. In WB4, addition of MEM in sub-inhibitory concentration (1/4 MIC) prevented the increase of the MIC for L over 10 cycles. In KR4, addition of MEM led to a twofold increase of the MIC for L after 10 cycles. The increase of the MICs of these four mutants can be explained by mutations in the genes responsible for quinolone-resistance.
Conclusions:
1. MEM and L act synergistically against penR pneumococci in vitro and in experimental meningitis.
2. Addition of MEM in subinhibitory concentrations (1/4 MIC) prevents L-induced resistance in vitro.

P1617 Comparative efficacies of moxifloxacin and levofloxacin against *Streptococcus pneumoniae* using a novel murine model of pneumonia

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Objective: Moxifloxacin is an 8-methoxy fluoroquinolone with enhanced activity against Gram-positive bacteria. Our aim was to compare the efficacies of moxifloxacin and levofloxacin in the treatment of pneumococcal lung infection using a novel murine model.
Methods: Groups of 19 immunocompetent Swiss Webster mice were infected by peroral tracheal instillation of 10^5 colony-forming-units of a *S. pneumoniae* serotype three strain. Antimicrobial treatment at a dose of 50 mg/kg was administered subcutaneously twice daily beginning at 24 h postinfection and continuing for 5 days. Skin-temperature was measured twice daily using an infrared temperature-scanning thermometer as a means of monitoring disease progression and severity. A drop of at least 3°C is predictive of imminent death in this model and was therefore used as the endpoint. Viable counts in the lungs of mice sacrificed and or surviving at the end of the study were determined.
Results: Moxifloxacin had a potent effect, reducing the pulmonary bacterial count to zero in all 19 mice. Skin-temperature changes of moxifloxacin-treated mice were negligible, as were uninfected mice, but were significantly different from untreated (mean, 4.8 ± 1.3°C) or levofloxacin-treated (mean, 5.1 ± 0.8°C) mice. In contrast to moxifloxacin-treated mice, 9 of the 19 levofloxacin-treated mice (47%) and 100% of the untreated-mice were either found dead or were sacrificed throughout the study. Viable counts (range, 10E+7 to 10E+8) were similar for both these groups, including two of the 10 levofloxacin-treated mice remaining at the end of the study. Resistant isolates were selected in six of the nine mice that failed levofloxacin therapy.
Conclusion: Moxifloxacin showed enhanced activity against *S. pneumoniae* compared with levofloxacin as suggested by its 100% reduction in pulmonary bacterial counts, its prevention of lethal changes in skin-temperature and its failure to select resistant bacteria.

P1618 Comparative activity between moxifloxacin and levofloxacin using an in vivo telemetric implant in a *Streptococcus pneumoniae* rat infection model

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Objectives: To monitor the course of a streptococcal infection by measuring the activity and temperature of rats using a telemetric implant and observe the comparative activity of two fluoroquinolones; moxifloxacin and levofloxacin.
Methods: Female Wistar rats (175–200 g) were implanted in the abdominal cavity with a VitalView PDT-4000 E-mitter and their postoperative recovery monitored by temperature and activity integrally every 15 min After a recovery period, animals were infected with *Streptococcus pneumoniae* 71B intraperitoneally. Immediately after infection, animals were orally administered with either levofloxacin or moxifloxacin at a dose equivalent to a C_{max} of 1.3 mg/L. Uninfected control animals and infected but untreated animals were included in the study.
Results: Uninfected control animals continued with their normal circadian rhythm of 24-h cycles with greatest activity and higher temperature seen in the 12-h periods of darkness. Infected animals however, had an interrupted circadian rhythm that saw an elevated temperature for 30 h ($P < 0.05$). The activity of this group was lower than uninfected controls ($P < 0.05$). Two infection control animals died; their initial temperature rise was for 6 h followed by a fall in temperature and their data was excluded from the statistical analysis. There were no deaths in the antibiotic-treated groups. Levofloxacin-treated animals showed a significant rise in temperature and loss of activity ($P < 0.05$) compared with uninfected controls. Moxifloxacin-treated animals had a small but not statistically significant temperature rise and lowered activity.
Conclusions: These data show that the streptococcal infection model is lethal to untreated animals. levofloxacin- and moxifloxacin-treated animals are afforded protection from death but not to the associated temperature rise and lowered activity. However, moxifloxacin has a significant effect in reducing the temperature and raising the activity associated with the infection compared with controls.

P1619 Establishment of an in vivo telemetric implant to monitor the course of a *Streptococcus pneumoniae* infection in rats

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Objectives: Animal models of infection and antibiotic treatment have mostly been quantified by cumulative death rates and clearance of microbes from the animal. This method has limitations for predicting antibiotic efficacy in humans as the infectious dose in most cases is by definition lethal. In this study, we have evaluated an implanted telemetric device that continuously gathers real-time information on an animal by measuring core body temperature (T) and activity (A).

Methods: Female Wistar rats (175–200 g) were implanted in the abdominal cavity with a VitalView PDT-4000 E-mitter and their postoperative recovery monitored by A and T integrally every 15 min After 5 days, animals were infected via the tail vein or intraperitoneally with a sublethal dose of *S. pneumoniae* 1707/4 and the course of infection monitored as described above. Animals were injected with buffered saline as an uninfected control groups.
Results: The period of full recovery from implantation until resumption of normal circadian rhythm was 30 h. At this point both A and T observed 24 h cycles corresponding to 12 h illumination/darkness with A and T higher in dark periods. No significant intra or intergroup differences in A and T were observed preinfection. Twenty-four hours postinfection, infected animals had a mean $T = 0.5–1°C$ higher than uninfected controls. The higher temperature lasted for 36 h ($P < 0.05$) until circadian rhythm was re-established. Mean A was reduced 30 h postinfection and remained lower for 60 h ($P < 0.05$). There was no significant difference between infection routes, however, intraperitoneal-infection was the preferred route as it was more rapid and caused less stress. The use of heat-killed bacteria did not have an affect on T or A. Uninfected control animals continued with normal A and T circadian rhythm during the experiment.
Conclusions: These data suggest that the continuous monitoring of body temperature and activity is a good guide to infection in a sublethal infection model. The 30 h window of infection will allow the monitoring of minor and early onset differences between efficacies of antimicrobial agents.

P1620 In vivo immunomodulatory profile of telithromycin in a murine infection model

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Objectives: In additional to their bactericidal activity macrolide antibiotics exhibit a broad spectrum of pharmacologic effects, which include immunomodulation. Telithromycin, a novel ketolide antibiotic is structurally related to macrolides. While the immunomodulatory effects of macrolides are well described, limited data exist with the ketolides. The objective of this study was to evaluate the immunomodulatory profile of telithromycin as assessed by five cytokines.
Methods: Specific-pathogen-free, female ICR mice were rendered transiently neutropenic with intraperitoneal cyclophosphamide. Thighs were inoculated with 106 cfu of a single isolate of *S. pneumoniae.* Once inoculated, mice were randomized to receive no treatment (controls); or single doses of 10, 25 or 50 mg/kg of oral telithromycin ($n = 30$ each). Blood was collected for cytokine determinations via cardiac puncture prior to and at 2, 4, 8 and 24 h after dose administration on five occasions. Cytokine (IL1-beta, IL2, IL6, IL10, and TNF-alpha) concentrations in serum were quantitatively measured with murine ELISA kits.
Results: While systemic concentrations of IL2 and TNF-alpha trended upward over the initial 8-h period post inoculation and a significant rise was noted with IL1-beta over 24 h, no marked immunomodulatory effects of telithromycin were seen for these cytokines. In contrast, significant elevations of IL6 and IL10 in untreated controls were noted over 24 h, while a correspondingly significant reduction of these two cytokines were noted after telithromycin administration. Additionally, the suppression of both IL6 and IL10 was observed to be dose-dependent.
Conclusions: While the dose of telithromycin in the current study was not optimized relative to human exposures currently under investigation in man, these data reveal the immunomodulatory potential of the ketolide, telithromycin.

P1621 Therapeutic efficacy of moxifloxacin or imipenem/cilastatin in experimental systemic aerobic/anaerobic mixed infection in mice

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Objective: To study the therapeutic efficacy of moxifloxacin (MXF) and imipenem/cilastatin (IMP) in severe systemic aerobic/anaerobic mixed infection in mice infected with both, different strains of *Bacteroides fragilis* and *Escherichia coli*.
Methods: A total number of 240 mice were divided in 12 groups each with 20 mice. Mice were infected intravenously with both, different strains of *B. fragilis* (IMP and MXF susceptible or resistant and/or enterotoxin positive or negative, respectively) and *E. coli* (IMP and MXF susceptible). Twenty-four hours postinfection, i.v. therapy with either MXF (2.0 mg b.i.d.) or IMP (2.4 mg t.i.d.) was started and continued for 3 days. Control groups were left untreated. Until day 7 postinfection, survival rates were observed. At day 7, postinfection surviving mice were killed and numbers of bacteria in liver and kidneys were determined.
Results: As compared with untreated animals significantly less mice died in the MXF or IMP treated groups. There was no significant difference in the survival rate comparing the two treatment arms irrespective of the infection with enterotoxin positive or negative strains of *B. fragilis* and the susceptibility to MXF and IMP, respectively. However, there was a tendency that *B. fragilis* was more often recovered from liver and kidneys of mice infected with enterotoxin positive strains.
Conclusion: Moxifloxacin was as efficacious as imipenem in the treatment of severe systemic aerobic/anaerobic mixed infection in mice.

P1622 An electron microscopic investigation of differential pathogenicities of various *Spiroplasma kunkelii* strains to the vector *Dalbulus maidis*

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Objectives: Spiroplasmas are mollicutes, and closely related to the human-pathogenic mycoplasmas, e.g. *Mycoplasma pneumoniae*, *M. genitalium*, and *Ureaplasma urealyticum*. Our research aims to understand the histopathologic basis of the interactions between pathogens and their vectors.
Methods: We undertook an investigation of pathogenicities of *Spiroplasma kunkelii* strains M2 and CS-2B to homopteran vector *Dalbulus maidis*. Insects fed *S. kunkelii* M2- and CS-2B-infected corn plants for 2 weeks were collected, and subjected to transmission electron microscopy process. Thin sections were stained with uranyl acetate and lead citrate, and immunolabeled with gold particles.
Results: Spiroplasmas entered *D. maidis* gut cells by endocytosis. They reached to the space between plasmalemma and basal lamina by moving through the cell. They predominantly proliferated in gut muscles, and degraded the basal lamina to move into the hemolymph. We observed two spiroplasmas connected to each other by a conjugation pilus in the hemolymph and numerous pili on the surface of *S. kunkelii* cells (see Fig. 1). Additionally, spiroplasmas were found in Malphigian tubules, skeletal muscles, nervous tissue, trachea, macrophages and female genital organs. Although numerous *S. kunkelii* M2 cells were seen in the cytoplasm and canaliculi of type III secretory cells of the salivary gland, any spiroplasmas could not be found in the salivary gland secretory cells of *D. maidis* infected by *S. kunkelii* CS-2B.

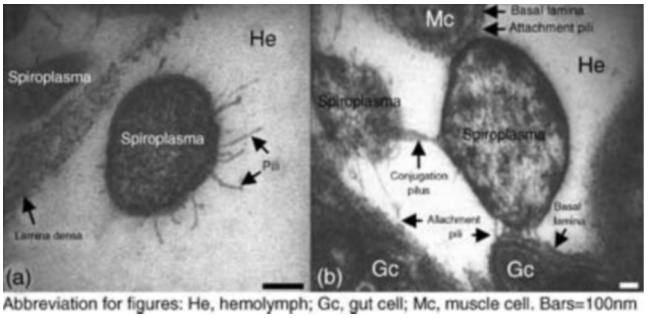

Figure 1

Conclusions: Pili are hair-like bacterial surface appendages (Low, D et al. 1996) that may facilitate pathogenesis, promote uptake or transfer of DNA or proteins, contribute to adhesion to specific receptors on various surfaces, and/or be involved in motility (Lai, E.M. and Kado, C.I., 2000). The absence of *S. kunkelii* CS-2B in salivary gland secretory cells suggests that the CS-2B strain is nontransmissible because it is unable to reach to the insect saliva. Besides, the results of our previous study (Bai, X et al. Evolution of Infectious Diseases Meeting 2002, Maryland, USA) showed that plant infection (or transmission) of *S. kunkelii* M2 was near 100%, whereas that of *S. kunkelii* CS-2B did not occur, and also some differences in *traE* gene expressions of *S. kunkelii* strains M2 and CS-2B. It is possible that *traE* genes affect transmission because they are associated with virulence.

P1623 Single dose Ceftriaxone for *Borrelia burgdorferi* infection in C3H mice

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Objectives: To determine the minimally effective therapeutic dose of the antibiotic ceftriaxone (CTX) for eliminating an active experimental infection with the Lyme disease bacterium, *B. burgdorferi* (Bb).
Methods: Using a mouse infectivity model for Lyme disease, recently published studies from our laboratory showed that five equally spaced doses of CTX, given over a 24-h period, were as effective as the more standard or routine 5-day treatment regimen with a single dose of CTX in eliminating viable Bb from mice infected with one strain of Bb. As a follow-up to these intriguing findings, in this study we next evaluated an even shorter course of CTX treatment in C3H mice infected with either of two different, North American-derived, strains of Bb *sensu* stricto. Separate groups of C3H mice (five per group) were infected intradermally with 100 000 culture-grown, low passage strain BL206 (mbc for CTX was 0.05 μg/mL) or reference strain 297 (mbc was 0.025 μg/mL) of Bb. Strain BL206 has been isolated from the blood of a patient having erythema migrans. Two weeks later, the mice were given a single intramuscular dose of either sterile isotonic saline or CTX (50 mg/kg). One week after treatment, extract cultures of the urinary bladder and ears were established in BSK media. These cultures were monitored weekly for motile Bb organisms based on dark-field microscopy.
Results: In infected control mice, live Bb organisms were readily isolated and culturable from extracts of the urinary bladders and ears. In contrast, it was found that the one-day, single dosage regimen of CTX was 100% effective in sterilizing the two selected tissue samples from mice infected with either strain of Bb.
Conclusions: These experiments suggest that shorter courses of antibiotics than those currently recommended should be considered for study in patients with early uncomplicated Lyme disease.

P1624 Efficacy of imipenem, sulbactam, and rifampin, alone and in combination, in experimental pneumonia caused by multiresistant *Acinetobacter baumannii*

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Objectives: To compare the activity of imipenem (IMP), sulbactam (SB), and rifampin (RF) in monotherapy and in combination in an experimental pneumonia murine model caused by *A. baumannii* (Ab).
Methods: In vitro studies: MICs and MBCs were determined in 16 bacteremic strains. The bactericidal activity and the synergy of the combinations (time-kill curves) were evaluated in four multiresistant strains. Pharmacokinetic/pharmacodynamic parameters (C_{max}, AUC, $t_{1/2}$, and time above the MIC) were determined after a single dose of each antimicrobial. In vivo studies: one strain resistant to all antimicrobials was used. The experimental pneumonia model in C57BL/6 mice was used, with an intratracheal inoculum of approximately 8 log10 cfu/mL. The animals were grouped in CON (controls, no treatment), IMP (120 mg/kg/day), SB (240 mg/kg/day), RF (100 mg/kg/day), IMP + SB, IMP + RF, and SB + RF, receiving treatment during 72 h. The mortality rate, the bacterial clearance from lungs and the sterilization of blood cultures were analyzed.
Statistical analysis: χ^2 and Fisher's test, ANOVA, and post hoc Turkey and Dunnett tests.
Results: In vitro: MIC/MBC (mg/L) ranges were as follows: IMP 32–128/32–128; SB 32–128/32–128; and RF 4–16/4–32. RF was bactericidal against all strains; IMP and SB were bactericidal against 2 and 1 strains, respectively. Synergy was found using the following combinations: SB + RF, IMP + RF, and IMP + SB, against 3, 2, and 1 strains, respectively. In vivo: The mortality of CON (69%) was decreased by IMP + SB (14%, $P = 0.006$) and RF (28%, $P = 0.03$), without differences of IMP (71%), SB (60%), IMP + RF (73%), or SB + RF (57%) with CON. The concentration of Ab in the lungs of the CON (log10 cfu/g: 8.1 ± 3.9) was decreased by RF (3.0 ± 1.9, $P = 0.01$) and IMP + RF (3.8 ± 2.1, $P = 0.04$); with IMP + SB (4.2 ± 2.7) and SB + RF (4.1 ± 2.2) there was a nonsignificant decrease; IMP (7.8 ± 3.4) and SB (7.2 ± 4.4) did not decrease the Ab concentration in the lungs. Respect to the sterilization of blood cultures, RF (78%, $P = 0.01$) was the only therapy better than the CON (27%), being also better than IMP and SB (31% and 33%, respectively, $P < 0.05$).
Conclusions: Monotherapy with rifampin or the combination of imipenem plus sulbactam are efficacious in the treatment of the experimental pneumonia caused by multiresistant *A. baumannii*.

P1625 Investigation of cytotoxic capacity of some adherent enterobacterial opportunistic strains by Mtt assay and transmission electron microscopy

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The purpose of this study was to determine the cytotoxic effect on Caco-2 intestinal cells, HEp-2 cells and rat macrophages of dialysates obtained from bacterial cultures of some enterobacterial opportunistic strains with different sources of isolation (food, stool culture, acute diarrhea, urine culture). The present strains were previously tested and selected for their intensive adherence and invasion capacity to the cellular substratum and also for their destroying effect on cellular monolayer. In this study the level of cytotoxicity was appreciated quantitatively by means of MTT assay and qualitatively by transmission electron microscopy (TEM). The MTT method is using a tetrazolium salt for the quantitative spectrophotometric assay of Caco-2 cells survival and proliferation rates in the presence of bacterial dialysates. This test detects the viable cells, which are able to reduce the tetrazolium salt and offers the advantages of a very simple, rapid and precise method. For the electromicroscopic examination the ultrathin sections obtained from samples containing bacterial cells incubated with HEp-2 cells and rat macrophages, respectively, were prepared following the standard protocol. Our results showed that the most cytotoxic strain proved to be *Enterobacter cloacae* 43 isolated from food, followed by *Citrobacter freundii* 93 strain isolated from stool culture and *E. coli* 115 strain isolated from acute diarrhea. These results correlate well with the electromicroscopic results pointing out the cytotoxic effect of *E. cloacae* 43 strain and its ability to induce A/E lesions in HEp-2 cells, as well as its apoptotic effect on rat macrophages. Besides their great adherence and invasion capacity, the production and elimination of cytotoxic factors in extracellular medium represent virulence factors in these strains and could be responsible for the increase of the pathogenic potential of opportunistic bacteria and explain their implication in the etiology of severe infections and food borne diseases. This study proved that the virulence of opportunistic pathogens is not correlated with the strain's origin, the most virulent strain being one strain of *E. cloacae* isolated from food.

P1626 Efficacy of Ramoplanin in the hamster model of *C. difficile* associated colitis

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Objectives: Ramoplanin (RA) is a novel glycolipodepsipeptide antibiotic endowed with potent in vitro bactericidal activity against all Gram-positive aerobes, including vancomycin-resistant enterococci (VRE), and anerobes, including *C. difficile*. RA is not absorbed by oral route and exerts its action locally in the gut. RA is presently in a Phase III trial being conducted by Genome Therapeutics Corp. for the prevention of VRE bloodstream infection in at-risk patients. Aim of the study was to evaluate the in vivo efficacy of RA in the hamster model of clindamycin (CL)-induced colitis in comparison with vancomycin (VA) or metronidazole (MZ).
Methods: (i) CL-induced colitis. Animals were treated with a single s.c. injection of 100 mg/kg CL, and after 24 h, received oral RA or VA at 50 mg/kg/day for 5 days. (ii) CL + *C. difficile* 4013 induced colitis. Animals were challenged orally with a bacterial suspension of *C. difficile* and treated 24 h after with a single s.c. injection of 100 mg/kg CL. Oral treatment with RA or VA at 25–50–100 mg/kg or MZ at 100–200–400 mg/kg started 24 h after CL and lasted for 5 days. Hamsters were weighed and observed every 24 h for evidence of diarrhea or moribund conditions. The cecal contents were analyzed for *C. difficile* toxin A (ELISA). Mortality was recorded for up to 36 days.
Results: (i) CL alone rapidly induced a fatal enterocolitis with 100% mortality within 4 days. Autopsy revealed hemorrhagic ceca and watery stools. *C. difficile* toxin A was always detected in these animals. Oral administration of VA or RA were highly effective in prolonging survival and protecting animals from death. RA was significantly more effective than VA with 80% survival rate compared with 20% of VA treated animals ($P < 0.05$). All hamsters that died had gross pathologic evidence of enterocolitis and cecal contents were

positive for Toxin A. (ii) The challenge with *C. difficile* to CL treated animals also induced a rapidly fatal enterocolitis. Both VA and RA were more effective in prolonging survival than MZ (13–17 and 9 days, respectively). Time of death of RA treated animals was delayed compared with that of VA treated animals, and 20% survival was recorded at the end of observation period in the 100 mg/kg group with RA. No animals treated in the VA or MZ groups survived.
Conclusions: The efficacy of oral RA in experimental *C. difficile* associated colitis warrant its clinical evaluation in the prevention and treatment of *C. difficile* associated colitis.

Pneumococcal and central nervous system infections in children

P1627 The Belgian IPD Survey: a one-year prospective survey of invasive pneumococcal disease in children under 5

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Objectives: To document the incidence of invasive pneumococcal disease (IPD) in Belgian children less than 5 years of age and to determine the serogroup and the antibiotic susceptibility of the pneumococci isolated from these children with IPD.
Methods: A one-year prospective surveillance from March 15th 2002–2003, in Belgian pediatric wards. Clinical data along with all pneumococci strains recovered from IPD cases were collected. Serotyping and antibiotic susceptibility testing (*E*-test) were performed by the Pneumococci National Reference Laboratory.
Results: After 9 months 204 cases of IPD were recorded. Meningitis was found in 15%, pneumonia with positive blood culture in 29%, occult bacteremia in 49%, empyema in 5% and arthritis or peritonitis in 2% of the cases. 73.5% of the IPDs occurred in children younger than 2 years. The serogroups included in the 7-valent conjugated pneumococcal vaccine (Prevenar(R)) accounted for 89% and 87% of IPD in children less than 2 years or less than 5 years, respectively. In the cases of meningitis the theoretical coverage of Prevenar was 92%. 13% of the pneumococci had a penicillin MIC > 0.06 μg/mL (none had a MIC ≥ 2 μg/mL). All of these pneumococci belonged to serogroups included in Prevenar and more than half were serogroup 14. 48.4% of the isolates were resistant to erythromycin and none to cefotaxim. For the full year, we expect an incidence of IPD of 48.3/100 000 and of pneumococcal meningitis of 7.3/100 000 in children less than 5 years old. This is higher than the incidence (30.2/100 000 for IPD and 4.3/100 000 for pneumococcal meningitis) calculated in 2000 based on spontaneous reporting to the Reference Lab. The incidence of meningitis is comparable to the incidence of *Haemophilus influenzae* b (Hib) infections before the vaccination era.
Conclusions: (1) IPD was common in children, with 75% of isolates in children under 2 years. (2) Pneumococcal meningitis had an incidence comparable to that of Hib infections before vaccination. (3) Inclusion of the Prevenar vaccine in the infant vaccination schedule could prevent 87% of all IPD in the studied age group. (4) Penicillin resistance in pneumococci was low.

P1628 Surveillance of antibiotic resistance and serotypes of *Streptococcus pneumoniae* in the Comunidad Valenciana (Spain)

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Objectives: The surveillance of the most prevalent serotypes (STs) of *Streptococcus pneumoniae* in our geographical area. Obtain an updated knowledge of *S. pneumoniae* antibiotic resistance in order to establish the most efficient antibiotic policy.
Methods: A total of 390 strains of *S. pneumoniae*, isolated from patients of different hospitals of the Comunidad Valenciana (June 1999–December 2001) were evaluated. STs were determined by Quellung reaction (reagents provided by Stantens Seruminstitut). MICs of oxacillin (OX), penicillin (PV), ampicillin (AM), amoxicillin/clavulanic acid (XL), cefuroxime (XM), cefotaxime (CT), erythromycin (EM), clindamycin (CM), rifampicin (RI), vancomycin (VA) and levofloxacin (LE) were determined by *E*-test method, and the breakpoints applied were NCCLS 2000 (M7-A5).
Results: 22.2% of *S. pneumoniae* were from CSF, blood and other sterile liquids. The most frequent STs were: 19 (23.3%), 6 (13.1%), 3 (11.8%) and 23 (11.5%). The percentage of highly resistant strains were: PV, 8.0%; AM, 0.9%; XL, 0.0%; XM, 3.2%; CT, 0.0%; EM, 46.5%; CM, 39.4%; RI, 0.0%; VA, 0.0%; and LE, 0.3%. Of the OX resistant (R) strains 81.9% were nonsusceptible (NS) to PV. The prevalent STs among the phenotype OX(R)-PV(NS) were: 19 (30.0%), 14 (15.8%), 6 (15.3%), 23 (13.7%) and 9 (10.0%). The percentage of strains with phenotype EM(S)-CM(S) were 52.6%. The 58.3% of these strains belonging to the STs 3, 19, 9 and 14. The percentage of strains with phenotype EM(R)-CM(R) were 38.0% and the most frequent ST were 19, 6, 23 and 14.
Conclusions: The 7-valent conjugated pneumococcal vaccine covers 78.3% of the strains from patients <2 years and the 23-valent polysaccharide vaccine covers >90% in patients older than 2 years old.

P1629 Invasive *Streptococcus pneumoniae* from infants: implications for vaccination in Portugal

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Objectives: Determine the serotype and antimicrobial susceptibility of invasive isolates of *Streptococcus pneumoniae* recovered from infants (less than 2 years of age) at the time of introduction of the 7-valent conjugated vaccine.
Methods: *S. pneumoniae* recovered from normally sterile sites during the years of 1999–2002 were sent to the Microbiology Laboratory of Faculdade de Medicina de Lisboa for study. Serotyping was done by the Quellung reaction using sera from the Statens Serum Institut (Copenhagen, Denmark). Susceptibility testing was performed by disk diffusion (according to NCCLS guidelines) and *E*-test. Antimicrobials tested included penicillin, cefotaxime, vancomycin, erythromycin, clindamycin, tetracycline, chloramphenicol and trimethoprim-sulfamethoxazole (SXT).
Results: The origin of the 57 isolates tested was: blood (50), CSF (5), other sterile fluids (2). Resistance to antimicrobial agents was detected against cefotaxime (1.8%), erythromycin (17.5%), clindamycin (7.0%), tetracycline (17.5%), chloramphenicol (7%) and SXT (35.1%). All isolates were susceptible to vancomycin. Penicillin nonsusceptibility was 45.6% (22 isolates low-level and 4 isolates high level, MIC50 = 0.032 mg/L; MIC90 = 2 mg/L). Simultaneous resistance to 3 or more antimicrobial classes was detected in 14.0% of the isolates. Penicillin nonsusceptibility was mostly (70%) associated with serotypes 14 and 23F found in internationally disseminated clones. Four serotypes accounted for more than half of all isolates: 14 (26%), 23F (16%), 6B (9%) and 19 A (9%). The remaining 39% of the isolates were distributed in 13 different serotypes. Three isolates from type 3 and two isolates each of types 9 V, 7F, 6 A, 33, 18, 10 and 1. Five isolates had unique types.
Conclusion: The 7-valent conjugated vaccine would cover at best 63% (considering cross-protection to serotype 6 A) of the cases of invasive disease among children younger than 2 years of age in Portugal.

P1630 Invasive Pneumococcus infections in an intensive care unit in the last 5 years

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Objectives: Our aim was to determine how many patients were treated in our ICU because of *Streptococcus pneumoniae* – induced invasive infection in the last five years. How long they stayed in the hospital, which age group was the most susceptible for the disease and which were the most frequent clinical forms of it. We also wanted to determine the susceptibility of Pneumococcus for penicillin therapy.
Methods: A retrospective study was made in the Intensive Care Unit of our hospital, collecting data of children having suffered from invasive Pneumococcal infection in the period of January 1 1998–December 31 2002. Our

criterium of diagnosing the disease was detecting Pneumococus from a normally sterile body fluid (pleural effusion, blood, liquor or abdominal fluid).
Results: During the last 5 years we treated 25 children with invasive Pneumococcal infection. The vast majority of them were suffering from pleural effusion (22/25) while there were also cases of meningitis, primary peritonitis and bronchopneumonia, one of each. Our patients were 3.1-year-old-on average (range 4 months – 12 years), the majority of them was female (14/25). None but one of them had had any history of either some chronic disease or previous severe infections. The average length of stay was 18.6 (range 3–40) days in our department and 24 days in the hospital altogether. We performed continous pleural suction to all our pleuropneumonia patients, for 9.6 (range 1–31) days on average and derived 790 mL (range 160–1920 mL) pleural fluid by using this method. We admitted most of the children in autumn, winter and early spring. We experienced some complications caused either by the disease or by the invasive tratment we had to use. Most frequent complications were respiratory failure, cardiac decompensation, pneumothorax, subcutaneous emphysema, atelectasia, pleural or mediastinal abscess. We treated one boy with hemolytic uremic syndrome and two children suffered from multiple organ failure. Pleural fluid culturing was positive in relatively few cases (13/25) while antigen testing revealed the bacterium more effectively (21/25). We cultured polyresistent pathogens from the pleural fluid relatively seldom (2/13).
Conclusion: The most susceptible age group to invasive Pneumococcal infection are infants. Administering conjugate Pneumococcal vaccine might be the really cost-effective solution in the long run.

P1631 Use of nasopharyngeal swabs isolates of *Streptococcus pneumoniae* from children in Sao Paulo, Brazil for surveillance of antimicrobial resistance

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Objectives: To determine whether antimicrobial susceptibility of SP isolated from the upper respiratory tract is representative of data from patients with invasive infections.
Methods: We collected Nasopharyngeal (NP) swab specimens from children, between 3 months and 5 years old. Those children attended the outpatient clinic of two hospitals in Sao Paulo City, between June 16, 1997 and May 20, 1998 (first period) and between March 15, 2000 and March 15 2001 (second period). The results of the surveillance were compared with data obtained from children admitted in hospital with pneumococal invasive infection (PII) in two periods: 1996–98 (101 patients) and 2000–2001 (40 patients). NP specimens were collected pernasally, using a calcium alginate swab and plated immediately after collection onto trypticose soy agar with 5% sheep blood and garamicin 5 μg/mL. Penicillin susceptibility of isolates was determined by a screening with oxacillin 1 μg disk and the minimal inhibitory concentration by the *E*-test. Serotyping was performed by the capsule Quellung method with use of antiserum for 46 serotypes.
Results: SP was recovered from NP of 139 children in the first period and 42 in the second period. The prevalence of penicillin nonsusceptible strains (PNS-S) in the NP carriage and PII was 16.4% and 17%, respectively, in the first period. In the second period PNS-S in the NP carriage was 42% and in PII was 45%. The serotypes 23F, 6, 14 and 19F were the most common in the NP study and serotypes 14, 1, 5 and 6 were the most common in invasive infections. Serotypes 6, 14 and 23F were the most prevalent among the PNS-S in both surveillance.
Conclusions: These findings suggest that nasopharyngeal isolates of *Streptococcus pneumoniae* from children with upper respiratory infections can be used to conduct surveillance for antimicrobial resistance in a defined geographic area but are not reliable for serotypes in our community.

P1632 Prospective study of bacterial meningitis in children

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From January 2000 to December 2002, we studied all bacterial meningitis cases with positive cultures, excluding possible contaminations, in children admitted in the Pediatric's department of our Hospital. The study included nine girls and 10 boys, ranging from neonates to 14-year-old-children. Cerebrospinal fluid (CSF) analysis and blood cultures were performed in every child at the admission time. Csf tests induced: cell count, glucose and protein levels, gram stain, latex agglutination tests for the detection of bacterial antigens, bacteriological culture and antimicrobial susceptibility tests. Chocolate agar and 5% sheep blood agar were used as isolation media for bacterial recovering. Blood cultures were performed in the Bactec fluorescent system 9120. Identification and antimicrobial susceptibility tests were both made in Vitek 1 automated system. Kirby-Bauer disk diffusion method was also used for the last tests. Glucose and protein levels of CSF was determinated in the Vitros System 250. In the 19 cases studied, the 20 isolated microorganisms were: *Neisseria meningitidis* – 12 (60%), *Streptococcus pneumoniae* – 3 (15%), *Haemophilus influenzae* – 2 (10%), group A streptococci – 2 (10%), group B streptococci – 1 (5%). The cell counts were elevated and segmented neutrophils cells predominated in 16 (84,2%) cases. The CSF glucose level was decreased in 9 (47.4%), the CSF protein level was elevated in 15 (78.9%), while the gram stain revealed the causative organisms in 16 (84.2%) cases. Rapid tests for bacterial antigens were performed in 13 cases, from which 4 were positive (30.8%) and 9 were negative (69.2%). Blood and CSF cultures revealed the same pathogen in 12 cases (63.2%). All gram-positive and gram negative microorganisms were sensitive to 3rd-generation cephalosporins. All gram-positive bacteria were sensitive to ampicillin, penicillin and vancomycin. 100% of the *H. influenzae* isolated were resistant to ampicillin. *N. meningitidis* was the most common pathogen found, followed by *S. pneumoniae*. The sensitivity of other than the cultural tests was good in a general way, however, the sensitivity of latex agglutination tests was low. Antibiotic sensitivity results were within the antimicrobial susceptibility patterns.

P1633 Mass vaccination campaign following an outbreak of serogroup C meningococcal disease in the Puy de Dôme, France, 2001–2002

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Objectives: to control an outbreak of serogroup C invasive meningococcal disease (IMD) in the Puy de Dôme, France, 2001–2002.
Methods: A case of IMD was defined as isolation of Nesseiria meningitis serogoup C from blood or CSF (using culture or PCR) in a resident of the Puy de Dôme. Cases were identified through an active notification system. Epidemiologic, clinical and biologic data along with the outcome under therapy were prospectively recorded. All strains were sent to the National Reference Centre for serotyping and multiloci DNA fingerprinting. In addition to a postexposure prophylaxis using rifampin, the health authorities decided a vaccination campaign targeting all infants children and young adults from 2 months to 20 years of age and living in the area where the cases were identified using a C monovalent conjugate vaccine (MeningitecR): 3 doses one month apart before 1 years (y) and 1 dose beyond this age.
Results: From February 2001 to January 2002, 18 cases of IMD (2.9/100 000p) occurred in the Puy de Dôme and 12 belonged to the serogroup C (2/100 000p): half of them occurred between November 2001 and January 2002. Half of the patients were children under 2y; 67% had a purpura fulminans; 75% were admitted to an ICU; the overall case fatality rate was 25%. The Reference centre identified a serotype 2a including 3 subtypes: P1-5 ($n = 6$), P1-2,5 ($n = 4$), P1-2 ($n = 2$); among those, one belonged to the ET-37 clonal complex. More than 64700 persons were vaccinated (around 90% of the targeted population) between 16th January 2002 (10 days after the last case of purpura fulminans) and 2nd February 2002. No severe side-effects were reported. One additional case of serogroup C IMD was diagnosed in August 2002 in a 17-year-old girl who had not been vaccinated because she was suffering from an infectious mononucleosis syndrome.
Conclusion: This first mass vaccination campaign in France, where the incidence of IMD is lower (1/100 000p) than in Northern European countries, is likely to have prevented new cases of serogroup C IMD in the area. Since then, other departments of Southern France faced a similar challenge and also decided to vaccinate the youngest population. The increasing incidence of serogroup C IMD in France together with vaccination policies implemented in several European countries strongly support a timely decision to protect the youngest population in France by using recently available C monovalent conjugate vaccines.

P1634 Third generation cephalosporins in the treatment of pediatric bacterial meningitis

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Objective: Evaluation of the treatment with third generation cephalosporins at the clinical course and complications of pediatric bacterial meningitis.
Patients and methods: The study is based on analyzing medical records of 403 children, aged 3 month to 14 years, admitted with diagnosis of purulent meningitis, during the period 1991–2001. The diagnosis of purulent meningitis is established on CSF pleocytosis with over 90% polimorphonuclear predominance, positivity of CSF and/or blood culture and/or elevated values of acute phase reactants. The patients were divided in two groups: the first one treated by the combination of ampicillin and chloramphenicol and second group where cephalosporin of third generation were used (ceftriaxone or cefotaxime). The dosage and treatment duration were applied according to recommended protocols. Clinical data included were: the duration of fever and meningeal syndrome, improvement of CSF data and incidence of complications.
Results: 125 children with mean age 3.07 years and 130 children with mean age 2.8 years belonged to the first study and second study group. Bacterial agents isolated At both groups were: *N. meningitidis* (36%), *S. pneumoniae* (34%), *H. influenzae* (20.4%). At 9.4% of cases, no bacteria were found. Mean fever duration was 3.81 days for the patients of the first group and 2.07 for those of the second group. Meningeal syndrome was present at mean range, respectively, 5.2 and 3.8 days. The improvement of CSF data was without any significant difference for both groups. Complications, such as hydrocephaly, psycho motor retardation, epilepsy, hearing impairment, were observed in 13.7% of the cases at the first group and 7.4% at the second group.
Conclusions: Cephalosporin of third generation have markly improved clinical course and complications of bacterial meningitis. The combination ampicillin and chloramphenicol still remains an effective, alternative treatment of pediatric bacterial meningitis.

P1635 *Haemophilus influenzae* type A meningitis following ahead injury

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A 7-year girl was admitted to the ICU of our hospital for fever and partial seizures. The physical examination revealed nuchal rigidity and right nostril anosmia. She required intubation and mechanical ventilation. Empiric therapy with cefotaxime IV, dexamethasone, and phenytoin was begun. A diagnostic lumbar puncture was performed. The CSF contained 1600 WBC/mm^3, protein of 177 mg/dL and glucose of 50 mg/dL. Gram-stain smear revealed pleomorphic Gram-negative bacilli. Detection of soluble antigen for *H. influenzae* type b (Hib) was negative. Culture of CSF grew *H. influenzae*. The strain was serotyped as type a, by agglutination in type specific antisera and as biotype V by apiNH. The organism was beta-lactamase negative. By the disk diffusion method, according to the NCCLS criteria, it was found to be susceptible to ampicillin, amoxicillin/clavulanate, cefotaxime, meropenem, clarithromycin, and trimethoprim-sulfamethoxazole. Blood cultures drawn on admission were negative. Initial laboratory evaluation revealed a WBC count of 14 900/mm^3, and a C-reactive protein of 12 mg/L, which was increased to 122 mg/L in two hours. An immunological investigation was unremarkable. In 2 days the child was much better, and transferred from the ICU to the pediatric ward. Past medical history revealed that 8 months before this episode, the child had been hospitalized in another pediatric hospital, after a car accident. Otorrhea had been observed. After pharmaceutical therapy she was discharged home in a good condition. Four months after the car accident she was hospitalized again because of pneumococcal meningitis. Axial cranial CT showed no bone lesions. There was a small collection of fluid in the sphenoid sinuses and in the right ethmoidal cells. After antibiotic therapy the child was discharged. The child had been completely vaccinated against Hib and *Neisseria meningitidis* type C. The head injury history, followed by two episodes of meningitis led the clinicians to ask for a new axial cranial CT, which showed no bone lesions, but fluid collection in the ethmoid, maxillary and sphenoid sinuses. Because of the existence of fluid in the sinuses, a high resolution cranial CT scan was performed, which revealed a fracture of the cribiform plate, with a fistula into the ethmoid. The patient was discharged after a 18-day cefotaxime treatment with the recommendation for vaccination against *Streptococcus pneumoniae*. She has since remained well, without antibiotic prophylaxis.

P1636 A case of bacterial meningitis by two simultaneous agents

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We report a simultaneous case of acute bacterial meningitis and bacteriemia by two differents agents, followed by septicemia by another agent. A previously healthy 17 months old child presented with fever, petechial exantema and prostration, following a five days history of diarrhea, fever and anorexia. The patient was admitted in the pediatric department and blood clinical chemistry, hematological, immunological and microbiological tests were made. Blood microbiological tests included: bacterial culture, antibiotic sensitivity and latex particle agglutination tests for the detection of bacterial antigens. Were also made analysis of cerebrospinal fluid (CSF), that included: cell count, glucose and protein levels, gram stain, latex agglutination test, bacteriological culture and antibiotic sensitivity. The blood cultures were performed in Bactec fluorescent system 9120. The isolation media for bacterial recovering were chocolate agar and 5% sheep blood agar. Identifications and antimicrobial susceptibility tests were both made in Vitek 1 automated system. The diffusion technique of Kirby and Bauer was used in tests of antibiotic sensitivity. The gram stain and latex agglutination tests were negatives, however, we isolated simultaneously from CSF and blood, *Haemophilus influenzae* and *Streptococcus pyogenes*. At the same time, imagiological tests (cranial CT scan, heart and abdominal ultra-sonography and chest roentgenogram) were performed and revealed no abnormalities. The tests of antibiotic susceptibility permitted continue treatement and after 9 days of treatment with ceftriaxone and ampicillin e.v., the CSF was sterile, but the child was febril and two blood cultures indicated a septicemia by *Pseudomonas aeruginosa*. Ceftriaxone treatement was stopped. The girl remained on ampicillin 4 days more and began gentamicine e.v. treatement. She recovered without sequelae after 20 days of hospitalization. The present case is very uncommon and we don't find any identical case reported in the reviewed literature. The laboratory diagnosis was essential for optimal therapy.

P1637 Spinal infection with *Actinomyces urogenitalis*

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Objectives: Spinal actinomycosis is a rare cause of central nervous system infection. We describe the case of a one year-old child with a sacral congenital dermal sinustract communicating with the dural sac that was complicated by infection with *Actinomyces urogenitalis*.
Methods: An 11-month-old-infant with recurrent meningitis underwent surgery for correction of a sacral durocutaneous fistulisation. Four days after the intervention the child became irritable, showed neurological detoriation and a bulging fontanel. The cerebrospinal fluid had a cloudy appearance. Spinal magnetic resonance imaging visualized the presence of an extensive lumbosacral abscess. In the cerebrospinal fluid and in the pus obtained during surgical drainage of the abscess a gram-positive rod was recovered. In addition to conventional bacteriological testing, partial DNA sequencing of the 16S rRNA gene was performed.
Results: Conventional bacteriological testing was compatible with the characteristics described in the literature for *A. urogenitalis*. The obtained sequence of 275 base pairs was compared with sequences available in GenBank database by using FASTA program. 100% identity with *A. urogenitalis* was found. Several other anaerobes were also isolated in the abscess: *Bacteroides ovatus*, *Finegoldia magna*, *Bacteroides urealyticus* and *Prevotella* species. The child was treated with high dose penicillin IV during 6 weeks followed by oral amoxicillin. However after four months of oral treatment the intraspinal lesions expanded again and reintervention was needed. Culture of the lesions did not grow any micro-organism. The patient was again treated with high dose penicillin for six weeks and subsequently with chloramfenicol orally. Until now (3 months later) the evolution of the patient is favorable.
Conclusions: We describe a case of spinal infection with *A. urogenitalis* that relapsed in spite of a prolonged oral therapy with amoxicillin. Diagnosis of actinomycosis was very important to take the decision to start a prolonged therapy with chloramfenicol.

Clinical aspects of tuberculosis and other mycobacterial infections

P1638 *Mycobacterium kansasii* infection with normal chest radiograph

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Objective: The aim of this work was to study the epidemiologic features of *M. kansasii* isolates in patients with normal chest radiograph
Patients and methods: We reviewed 334 records of patients with *M. kansasii* isolated from culture specimens from 1994 to 2002. We applied ATS diagnostic criteria for nontuberculous mycobacterial disease to the cases and then we utilized modified criteria in defining all of our cases. One of them was the presence of several isolates from the respiratory tract with compatible respiratory symptoms together with a normal chest radiographic. Different molecular typing methods including PCR-RFLP and AFLP analysis were applied to clinical isolates of *M. kansasii*.
Results: During the study period 36 patients had a normal chest radiograph: 13 (36.1%) HIV positive and 23 (63.9%) HIV negative patients with a male predominance 4/1 and a mean age 56.50 range [26–88 years]. Only in 12 (33.3%) of HIV negative patients the Chest X-ray show abnormalities of a COPD. Respiratory samples of 18 patients (50%) were acid-fast smear positive, 10 patients were HIV positives and 8 HIV negatives. Multidrug regimen containing isoniazid, rifampicin and ethambutol was initiated in 31 patients (86.1%). Five HIV negatives patient didn't receive treatment; two of them died when the microorganism was recovered. Recurrence of clinical disease occurred in only one patient HIV negative. Five HIV positive patients had disseminated disease, the mycobacterium were recovered from stool, respiratory, pus, and urine samples. PCR-RFLP analysis demonstrated that the type I was the most prevalent *M. kansasii* (16/17) isolates and only one isolate was type II. AFLP analysis of type I strains revealed five clones although two of them were the most prevalent.
Conclusions: The number of patients with *M. kansasii* in respiratory samples with a normal chest radiograph is important and not only in HIV positives patients where this issue has been described, also is frequent in our cases in HIV negative patients. The number of acid smear positive is also elevated. Isolation of *M. kansasii* from the respiratory tract in HIV positive prompt initiation of antimycobacterium therapy but this doesn't occur with HIV negatives.

P1639 Impact of migration on the prevalence of mycobacterial infections in HIV-positive patients in Ireland: 1999–2002

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Background: With the advent and availability of HAART it was expected that the prevalence of mycobacterial infections in HIV+ patients would decline precipitously. However changing demographics in the HIV+ cohort in Ireland resulting from increased non-national migration has altered the presentation of mycobacterial infections. In 1999, less than 10% of new HIV diagnoses were recent migrants from Subsaharan Africa compared with 36% in 2000, 41% in 2001 and 44% in 2002.
Methods: A retrospective analysis of pharmacy records was undertaken to look at the prescription of antimycobacterial medication to HIV seropositive persons attending our service from January 1999 to end December 2002. Information was collected regarding nationality, mycobacterial species, site of infection & drug regimen prescribed. HIV parameters were also recorded. Patients receiving antimycobacterial chemoprophylaxis were excluded from the analysis.
Results: Forty-five cases of mycobacterial infection were identified in 42 patients. Twenty-one of 42 were from EU states, 21/42 were non-EU nationals. Twenty-six of 45 cases were *Mycobacterium tuberculosis* complex (MTBC), 4/45 cases presumptive MTB (PMTB), 11/45 cases were confirmed Mycobacterium other than TB (MOTT), 4/45 cases were treated empirically as MOTT. One MTBC isolate was multidrug resistant (MDRTB). Fifteen of 42 patients had CD4 counts <50; in this group were six cases of culture confirmed MOTT, two further were treated empirically, seven confirmed cases of MTBC and one case of PMTB. Twenty-seven out of 42 patients had CD4 counts >50; in this group were 19 cases of MTBC, 3 were PMTB; 5 cases had MOTT confirmed (clinically interpreted as infection rather than colonization), 2 patients were empirically treated as MOTT. Non-EU nationals comprised 33% of MTB cases in 1999 and 2000 (1/3 and 2/6, respectively); 50% (3/6) in 2001 but 80% (12/15) of cases in 2002.
Conclusion: In the era of HAART, nationality now impacts on the prevalence of MTB infection in HIV seropositive persons.

P1640 *Mycobacterium xenopi* disease in HIV-infected patients in the era of highly active antiretroviral therapy (HAART), compared with the pre-HAART period

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Objective and methods: Aim of our survey is to report the epidemiologic, laboratory, clinical, and therapeutic features of all confirmed HIV-associated *M. xenopi* disease observed 1993–2002, with special attention paid for differences raised after HAART introduction.
Results: Seventeen consecutive episodes of *M. xenopi* were retrieved in 3022 HIV-infected patients in our 10-year experience, so that a 0.56% frequency of *M. xenopi* disease was found. Our 17 episodes involved 14 patients: 2 suffered from 3 and 2 relapses (5 and 6 years after the 1st episode in the 1st patient, and 18 months later in the 2nd patient). No time- and space-clustering was recognized. The majority of *M. xenopi* diseases (15) involved the lower respiratory tract, followed by urinary and lymph node localization. An advanced HIV disease/AIDS and a low CD4 count (mean 42 cells/μL) prompted *M. xenopi* infection, although the pathogenic role of *M. xenopi* was difficult to separate from that of coinfecting organism (present in 34% of p). Most of our patients (76.5%) did not undergo HAART prior to diagnosis. Comparing the 13 episodes occurred before HAART with the 4 cases diagnosed in the HAART period, no significant difference was found as to age, gender, HIV exposure, AIDS diagnosis, infection site, number and type of concurrent AIDS-related illnesses, and frequency of relapses, but a higher mean CD4 count was seen in HAART-treated p ($P < 0.001$), as well as lung cavitations accompanied by exudation, which were never found in absence of HAART ($P < 0.006$). These features occurred in p who had a rapid recovery of CD4 count (280–700%), and should be a part of the immune reconstitution syndrome. Chemoprophylaxis protected 75% of p against *M. xenopi* disease when HAART was administered, while this rate dropped to 23.1% when HAART was not used. In vitro sensitivity assays identified ethambutol, cycloserine, capreomycin and protionamide as the most effective anti-*M. xenopi* drugs (100% sensitive strains), followed by isoniazid and rifampicin (94.1%), PAS, kanamicin, and streptomycin, in absence of temporal changes. Clinical-microbiological cure was reached in 76.4% of cases.
Conclusions: A reliable and timely clinical and bacteriological diagnosis, and an optimal treatment of atypical mycobacteriosis remain a challenge for clinicians facing immunocompromised p. Diagnostic problems posed by late identification due to slow culture and concomitant opportunism, join therapeutic difficulties due to the unpredictable in vitro susceptibility profile of these organisms.

P1641 Tuberculosis in HIV-1 infected children

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Background: (i) To assess the prevalence of *mycobacterium tuberculosis* (mt) infection in HIV-1 infected children from Mulago Government Hospital Pediatric clinic. (ii) To study the clinical and immunological status of the cases confirmed by culture and bacterial resistance to antituberculosis drugs.
Methods: Prevalence of TB was evaluated in a retrospective study based on the clinical records of HIV-1 infected children monitored in Mulago hospital pediatric clinic during a 10-year period of time (1991–2001). Diagnosis of TB was stratified as presumptive (clinical and radiological data) and definitive diagnosis (positive culture). The patients were classified according to the CDC classification for children.
Results: From 1176 patients, diagnosis of TB was established in 342 (24.6%) cases. Two hundred and eighty-six (85.2%) had pulmonary TB and 31 (9.8%) extra pulmonary/disseminated TB. For the 41 (17.2%) patients (Sex ratio 28 : 13) confirmed by culture, the average at the time of TB diagnosis was

8 years and 5 months. The transmission root was parenteral in 36 (87.8%) cases and mother to child in 5 (12.2%) cases. At the moment of TB diagnosis, 5 (12.2%) patients were stabilized in class A, 14 (34.1%) in B and 4 (9.7%) in C. According to the immunological status, 4 (9.7%) patients were classified: class 1.6 (14.6%) to class 2 and 13 (31.7%) to class 3. Radiology revealed hilar adenopathy (10 cases), infiltrates:alveolar (11 cases), interstitial (4 cases).
Conclusion: Susceptibility to antituberculosis drugs was determined in 10 cases, revealing mt resistance to isoniazid (INH) in 9 cases (90%), for rifampin (RMP) in 5 (50%) cases, for streptomycin (SM) in 4 (40%) cases.

P1642 The incidence of TB pericarditis in patients with acute pericardial disease in two years

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Background: Although during the last decades the prevalence of TB has declined and its prognosis has changed dramatically owing to chemotherapy cases of TB pericarditis can still be observed. Difficulties in early and accurate diagnosis probably contributed to this phenomenon and may delay diagnosis and therapy or lead to antituberculous chemotherapy for idiopathic pericarditis. So in this report we studied patients in whom a diagnosis of TB pericarditis was made by following Adenosin Deaminase Activity and other procedures.
Patients and method: We now report our experience in a descriptive group of 30 cases (19 men, 11 women), aged 12–76 years admitted to the hospital for acute pericardial disease. The diagnosis of TB pericarditis was made by the following studies: Culture on Lowenstein-Jensen media, Ziehl-neelsen staining, measurement of ADA of pericardial fluid, biopsy of pericardial tissue and evaluation of tuberculin test.
Result: We found 13 patients with TB pericarditis. All of them had clinical TB manifestation. The culture of pericardial fluid in six patients (46%), the staining in three cases (23%), the culture of pericardial tissue in 6 patients (46%) were positive. The caseating granuloma in 46% of cases was identified. Ten case (77%) had positive PPD reaction. All of them with increased ADA (>45 μ/L) were reported.
Conclusion: Therefore the evaluation of level of ADA may prove a good screening for TB pericarditis.

P1643 Tuberculous meningitis: retrospective study of 26 cases

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Objective: To review the clinical manifestations, laboratory, radiological findings and prognostic factors of patients with tuberculous meningitis (TM), admitted to our clinic.
Methods: Twenty-six patients with TM hospitalized in our clinic between January 1998 to January 2003 were evaluated retrospectively. Primary parameters were history, physical examinations, routine laboratory and cerebrospinal fluid (CSF) results, cranial tomography (CT) findings with clinical prognosis, complications, treatment and prognosis.
Results: The most frequently observed symptoms and/or clinical signs of the patients were headache (100%), meningeal irritation findings (80.8%), fever (65.4%). Acid-fast bacilli were seen in the CSF samples of 2 (7.7%) cases with Erlich-Ziehl-Neelsen method, *Mycobacterium tuberculosis* were isolated from 2 of 26 on Löwenstein-Jensen medium. Nine of 26 patients (34.6%) died. The cases who resulted in death were at phases II and III.
Conclusion: The most important factors affecting the prognosis in TM were the time between the development of symptoms and treatment as well as the neurologic phase of the disease.

P1644 Thwaites' diagnostic scoring and prediction of tuberculous meningitis

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Objective: Rapid and accurate clinical evaluation is required to determine the type of meningitis. This study was conducted to study applicability of Thwaites' diagnostic criteria in order to differentiate tuberculous meningitis from bacterial meningitis.
Methods: The study was held retrospectively in HIV-negative 126 patients with meningitis. One hundred and two of the patients had bacterial meningitis and 24 patients had tuberculous meningitis. The diagnosis was confirmed microbiologically in 59 and 12 patients with bacterial and tuberculous meningitis, respectively. The prediction of tuberculous meningitis was determined by Thwaites' diagnostic scoring using parameters such as, age, history of illness, white blood cell count (WBC), total cerebrospinal fluid (CSF) white cell count and the percent of neutrophils in CSF. The diagnostic value of the model was assessed by calculating the area under the receiver operating characteristic (ROC) curves.
Results: The sensitivity and the specificity of Thwaites' diagnostic scoring were 95.8% (23/24) and 71.6% (73/102), respectively. In microbiologically proven cases the sensitivity and the specificity were 91.7% (11/12), 79.7% (57/59), respectively. Area under ROC curve was 0.92. The area under ROC curve value for the diagnostic scoring was 0.92.
Conclusions: It was concluded that Thwaites diagnostic score is helpful in differential diagnosis of tuberculous meningitis and the usefulness of the diagnostic scoring should be validated in large series especially in patients with viral meningitis and low CSF glucose levels.

P1645 Molecular epidemiology of *Mycobacterium tuberculosis* from a pediatric population and comparison with an adult population

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Objectives: To provide details of the genotypes and clonal distribution of *Mycobacterium tuberculosis* (MTB) from the pediatric population, and to compare this data with the available genotyping profiles of MTB isolates from the adult population in our health district.
Methods: MTB isolates of patients under 15 years of age were selected over the last 13 years. Molecular typing was performed by spoligotyping. The typing patterns were compared with those obtained from a selection of the adult population from the same period. Medical histories and contact investigation records were examined to determine epidemiologic links.
Results: During the period of study (January 1990–May 2002), 68 culture-confirmed cases of tuberculosis in children were identified and 50 isolates were available for typing. Twenty-nine different spoligotypes were obtained, 58% of which were shared by the adult population. Sixty percent of the pediatric isolates were clustered, similar to the proportion found in the adult population (65.8%). All but one of the pediatric isolates in clusters were also found in the adult population. Pediatric clones were homogeneously distributed throughout the study period and no epidemiologic link was found among the clustered isolates, except for three isolates that constituted a microepidemic.
Conclusions: The molecular features of MTB isolates in pediatric infections, where tuberculosis is due to recent transmission, match those of the adult population: (i) there is a high variability of strains (ii) the composition of clones does not vary throughout the study period (iii) an equivalent percentage of typing profiles are clustered (iv) the composition of strains is similar. These equivalencies in the general molecular parameters of MTB isolates from children and adults could suggest that recent transmission of MTB plays a significant role in both populations.
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P1646 Preference for extra-respiratory dissemination by *Mycobacterium tuberculosis* correlates with highly efficient infection of macrophages in vitro

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Background: We have previously reported cases of coinfection by two different *Mycobacterium tuberculosis* (MTB) strains with different strains infecting at respiratory and extrarespiratory sites (compartmentalization of the infection). In one of these cases, two different MTB strains were isolated from the respiratory sample but only one of these two strains was found at the extrarespiratory site. This suggests clonal selection between the respiratory strains in order to disseminate to extrarespiratory sites.

Objective: To explore the role of strain factors which could be related to the ability of MTB to disseminate to extrarespiratory sites.
Methods: Two MTB strains (A and B) isolated simultaneously from a respiratory sample (only strain A was recovered from the extrarespiratory sample) were selected for study. Macrophages were cultured from monocytes obtained from the peripheral blood of healthy donors. The two respiratory strains were used to coinfect simultaneously (A:B proportions: 1 : 1, 10 : 1 and 1 : 10) macrophages in vitro. After 24 h, infected macrophages were lysed and plated to obtain single MTB colonies. Fifty single colonies were analyzed by spoligotyping to study the proportion of strains A and B after macrophage infection.
Results: Strain A infected macrophages more efficiently in the coinfection assay. After macrophage infection, the proportion between A and B strains was 24 : 1, 50 : 1, 1 : 3 for the assay proportions 1 : 1, 10 : 1 and 1 : 10, indicating that strain A infected macrophages more efficiently than strain B.
Conclusions: A phenomenon of competition to infect macrophages was detected between two strains that were coinfecting a patient at the respiratory site. The strain that showed a preference for disseminating to the extrarespiratory site in this patient was also able to infect macrophages in vitro more efficiently. These results suggest that (i) not all MTB strains disseminate with equal efficiency and (ii) strain factors such as infectivity could play a role in controlling extrarespiratory dissemination.

P1647 Sputum smear conversion during antituberculous therapy of alcoholics with pulmonary tuberculosis

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Objectives: The purpose of this study was to determine whether smear conversion correlates within the first month and sixth month of therapy at alcoholic patients with pulmonary (TB).
Methods: Forty-five alcoholic and 45 non alcoholic, totally 90 patients were studied. AFB smear of each patients were evaluated for the first month of therapy and at the end of the sixth month. Alcoholism was determined by using CAGE test.
Results: Following initiation of antituberculous therapy, 21 (38%) alcoholic and 34 (62%) nonalcoholic patients (group 1, responders) had a complete response to treatment and the amount of acid-fast bacilli (AFB) in the smears of the patients sputum decreased steadily, during the hospitalization period. The remaining 24 (69%) alcoholic and 11 (31%) nonalcoholic patients (group 2, nonresponders) had persistent evidence of active disease and demonstrated little or no decrease in the amount of AFB with treatment. At the follow-up of self-administered therapy period, 16 (36%) (7 in group 1, 9 in group 2) from alcoholic and 8 (18%) (7 in group 1, 1 in group 2) from nonalcoholic patients, totally 24 (27%) patients were excluded from the study that they had no regular visits to TB centers and no data available for these patients. At the end of the sixth month, 18 (62%) (12 in group 1 and 6 in group 2) alcoholic (n:29) and 30 (81%) (24 in group 1 and 6 in group 2) non alcoholic (n:37) patients had a clearance of AFB from sputum smear whereas 11 (38%) (2 in group 1 and 9 in group 2) alcoholic and 7 (19%) (3 in group 1 and 4 in group 2) non alcoholic patients had persistently positive AFB smears. Moreover, multidrug resistance was determined at 7 (39%) (1 in group 1 and 6 in group 2) alcoholic and 4 (22%) (1 in group 1 and 3 in group 2) non alcoholic, totally at 11 (61%) patients that had persistence positive AFB smears (n:18). The predictive value of smear negativity for the patients at the end of the sixth month at group 1 was found to be 86% for alcoholic and 89% for non alcoholic patients, whereas for negative predictive value the ratio was 60 and 40%, respectively.
Conclusions: AFB smear conversion in the first month of the therapy has a relation with the AFB smear negativity at the end of the sixth month of therapy. Given the relationship between this parameter, the model may be of value in the evaluation of new tuberculosis therapeutics in alcoholic patients with pulmonary TB, as well as in the care of individual patients.

P1648 Polyclonal primoinfection by *Mycobacterium tuberculosis*

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Objectives: Mixed infection by different *Mycobacterium tuberculosis* (MTB) strains has been described in recent years, and is usually assumed to be due to superinfection of an uncured episode with a second TB strain. Our aim is to explore whether polyclonal infections by MTB can also be caused by primoinfection by two different strains simultaneously.
Methods: MTB-positive respiratory cultures from children were selected in order to study patients with documented primoinfection. MTB isolates were plated onto solid media to obtain single colonies. Thirty independent colonies from each case were analyzed by spoligotyping. IS6110 RFLP was performed to confirm typing results when necessary.
Results: During a two-year period, 12 children with MTB respiratory isolates were available for study. For 11 cases, all the 30 colonies analyzed from each patient showed a unique spoligotyping pattern, indicating infection by a single MTB strain. In one case, two different spoligotyping patterns were obtained (proportion 24 : 6), indicating dual infection by two different MTB strains. Typing by RFLP confirmed this result. This case corresponded to a 2-year-old-child with no risk factors for tuberculosis.
Conclusions: In our population, simultaneous infection by more than one MTB strain was found in 8% of patients with documented tuberculous primoinfection, as confirmed by molecular typing. From this finding, it can be inferred that primoinfection in tuberculosis should not be assumed to be caused always by a single MTB strain, even in cases with no risk factors for TB.
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P1649 Demographic, social, and clinical characteristics of patients diagnosed with tuberculosis and admitted to an isolation unit

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Objective: To assess the demographic, social, and clinical characteristics of tuberculosis in patients admitted to the Isolation Unit during the comprising period 1998–2001.
Methods: Record files of 475 patients admitted to the Unit during the period 1998–2001 were evaluated, since their admission to the final follow up. Data were double-checked by the Epidemiology Department of the CM.
Results: Tuberculosis was the final diagnosed in 372 patients (78.3%). Mean age was 40.7 years old (SD 16.75), 68.8% of them were men. From the total of patients, 65,1% had been translated from the emergency room and 18% of them came from other hospital wards. Up to 27, 7% of inpatients were foreign-born, 46% of them originally from Latin America and 19.4% from North of Africa. Some risk factors associated with the diagnosis of tuberculosis were found in 66.3% of the patients: homeless (16.4%), alcohol abusers (27.7%), HIV positive (17.7%), drug users (16.9%) and previous contact to patients with active tuberculosis (21.2%). Concomitant diseases were present in 54.4% of patients, with HIV infection in 15,3% of patients and liver disease in 14.5%. Tuberculosis was located in the lungs in 98% of cases, 5.4% of them complained of constitutional symptoms, 11.9% of respiratory symptoms, 74.7% had both of them and 8.1% presented no symptoms. Microbiological data reported positive mycobacterium staining at admission in 87.4% of patients, positive mycobacterium cultures in 90.1% of cases. Sensitivity tests were performed on 79.6% of cases, 5.1% of them with multidrug resistant strains and 4.1% with resistant to some of the drugs. Side-effects to the antituberculosis drugs occurred in 28.5% of patients under treatment. Final results obtained through the study reported as cured 53.2% of patients, 12.6% completed treatment, 7.5% of patients were exitus and 23.9% of patients were lost during the follow up.
Conclusions: In this study, the patients diagnosed of tuberculosis were mainly young males, over a quarter were foreign born, two thirds of the cases presented some risk factors and over half of them presented concomitant pathology at the time of diagnosis. Pulmonary tuberculosis was the primary affectation, with positive smear tests and with a moderate rate of multidrug resistance strains. Finally, although the overall rate of cure was high, the rate of patients lost in the follow up was high as well.

P1650 Fever of unknown origin: a review of 51 patients with tuberculosis

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Objectives: Fever of unknown origin (FUO) is a challenging problem for the practising physician all over the world, and requires both knowledge and experience. The criteria includes: fever of more than three weeks' duration,

documented fever higher than 38.3°C on several occasions, and uncertain diagnosis after one week of examination and investigation in the hospital. In this study, we aimed to determine the clinical features of the patients with tuberculosis admitted with FUO and their rate to all of the patients with FUO during last 19 years in our unit.
Methods: Between 1984 and 2002, the patients with FUO and their etiology were determined by their files. The files of FUO patients with tuberculosis were thoroughly investigated. The diagnosis of tuberculosis was established according to the radiological and/or microbiological and/or histopathological and/or clinical response to anti-TB therapy in each clinical form. Ehrlich-Ziehl-Neelsen staining was used to determine acid-fast bacilli in clinical samples (fluid or solid). *M. tuberculosis* was cultured in Lowenstein-Jensen media.
Results: During last 19 years, 162 patients were followed with FUO and 65 (40%) had tuberculosis. TB constitutes 78% (51/65) of all infectious etiologies. Out of TB cases, 8 were pulmonary TB (1 reactivation, 7 primary), and 43 were extrapulmonary. Extrapulmonary tb forms were: miliary ($n = 21$), mediastinal TB lymphadenitis (8), disseminated TB (5), renal TB (3), systemic TB lymphadenitis (2), primary hepatic TB (1), TB peritonitis (1), Pott's disease (1), and mesenteric TB lyphadenitis (1).
Conclusion: Infectious diseases were found to constitute 40% of the FUO etiology, TB being the majority (approximately 3/4) among infectious ones.

Emerging infections: microbiology and diagnostics

P1651 VTEC O117:K1:H7: a new clonal group of diarrheagenic *E. coli* causing chronic travelers' diarrhea

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Objectives: We describe findings of 20 patients with Verocytotoxin-producing *E. coli* (VTEC) strains of serotype O117:K1:H7 during a 5-year period and present molecular and conventional phenotypic characteristics.
Methods: Clinical data were obtained by interview of patients. Stools were examined for Verocytotoxin-producing *E. coli* (VTEC) by colony dot blot hybridization with virulence gene DNA probes and the Vero cell assay. Serotyping was done by microtiter plate- and tube agglutination and production of hemolysin on washed sheep blood agar. Biotyping included 14 carbohydrates. Antimicrobial susceptibility testing towards 11 antibiotics employed Rosco Neo-Sensitabs. PFGE profiling was performed using XbaI enzyme according to the PulseNet US conditions.
Results: By PFGE, the strains were clonally related and they possessed similar unusual biochemical markers indicating that VTEC O117:K1:H7 is a clonal group of diarrheagenic *E. coli* not previously described. All strains were positive for the *vtx1* gene and negative for the *eae* gene. Eight strains (40%) were multiresistant. Eighteen patients had traveled to Asia, Africa or Cuba. The duration of symptoms was median 11 weeks, range 2–100 weeks. Twelve out of 15 (80%) patients had symptoms of diarrhea lasting 30 days or more (=chronic infection). Two patients were healthy carriers. No fatal cases occurred and none developed HUS.
Conclusions: To our knowledge, this is the first time a chronic infection with VTEC has been described. We recommend considering the VTEC O117:K1:H7 clonal group when diagnosing patients returning from Africa and Asia, particularly when presenting with long-lasting watery diarrhea.

P1652 Characterization of toxigenic *Corynebacterium ulcerans* strains isolated in the UK from humans and domestic cats

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Objectives: *Corynebacterium ulcerans* is a veterinary pathogen and causes mastitis in cattle and other domestic and wild animals. Toxigenic strains of *C. ulcerans* have been associated with classical diphtheria as well as milder symptoms. At least one death within the UK has been attributed to such an infection. Usually human infections are acquired through contact with animals or by ingestion of unpasteurized dairy products. Person-to-person transmission of *C. ulcerans* could be possible and it has been recommended that the public health response to human infection with *C. ulcerans* should be the same as for *C. diphtheriae*. In the UK, the frequency and severity of *C. ulcerans* infections appear to be increasing. Between 1986 and November 2002 a total of 55 *C. ulcerans* isolates were submitted to the PHLS Streptococcus and Diphtheria Reference (SDRU) Unit for identification. Forty-nine (89%) of the isolates were toxigenic. Recently, toxigenic *C. ulcerans* has been isolated from three cats with bilateral nasal discharge. The organism had not been identified previously in cats and our ribotyping data have shown that the profiles produced by isolates from the cats are thus far, indistinguishable from the two predominant patterns observed amongst human isolates. 16S rRNA sequencing and other tests were undertaken to confirm both the species identification and other characteristics.
Methods: A total of 68 isolates of *C. ulcerans* (55 clinical isolates from the UK, 3 isolates from 3 cats in the UK and 10 overseas clinical isolates) were characterized by biotyping, toxigenicity testing and ribotyping. 16S rRNA gene sequencing was also carried out on the three isolates from the three cats to confirm its species identification.
Results: Ribotyping revealed nine distinct ribotypes amongst the 65 clinical isolates from the UK and overseas. Ribotype profiles produced by isolates from the cats have been documented amongst human clinical isolates and 16S RNA gene sequencing confirmed the species identities of the strains from the cats as *C. ulcerans*.
Conclusions: *C. ulcerans* has not previously been reported in cats; however, our results suggest that the organism in cats could be a potential source of human infection. The source of human *C. ulcerans* infection in the UK is not often determined and this study highlights the importance of determining the source of infection. This is one of the objectives of the European Commission DG SANCO project 122/SID/2001.

P1653 Dry-heat inactivation of parvovirus B19: evaluation of a new LightCycler RT-PCR infectivity assay for detection of infectious B19

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Objectives: Parvovirus B19 (B19) is a widely distributed infectious agent, which causes variety of illnesses including erythema infectiosum (fifth disease) in children and arthritis, aplastic crisis and hydrops fetalis in pregnant women. B19 can be transmitted from asymptomatic blood donors to the recipients of their blood components. High rates of seroconversion, as well as a few cases of symptomatic illness, have been described in patients receiving RBCs, platelets, solvent-detergent (SD) treated plasma and clotting factor concentrates derived from plasma pools. Recently a new fibrin sealant product, hemostatic dressing (HD), has been developed by the American Red Cross (USA), CSL (Australia) and ZLB (Switzerland). HD, a unique combination of two active plasma components (fibrinogen and thrombin), rapidly forms a strong fibrin 'clot' upon contact with blood in a wide range of surgical procedures. We have evaluated two viral inactivation steps, SD treatment for inactivation of enveloped viruses such as HIV, HCV and HBV, and dry-heat treatment for inactivation of both enveloped and nonenveloped viruses such as HAV and B19 for inclusion in the manufacturing process. However, due to the lack a suitable cell culture system and an assay for quantitative analysis of the infectivity of B19, the effect of dry-heat treatment on the inactivation of B19 has not been investigated in the past.
Methods: Dry-heat treatment was performed for the fibrinogen component of HD spiked with B19 at 100° for 0, 1, 2 and 3 h. Real time (LightCycler) RT-PCR infectivity assay was developed and used for analysis of B19 samples.
Results: B19-specific LightCycler RT-PCR infectivity assay exhibited rapid and reproducible quantification of infectious B19. The assay designed on detection of spliced RNA forms of B19 in infected cells may not be affected by the presence of high amounts of viral DNA. Both a standard of infectious B19 and a test of B19-specificity are established. Unique primers are designed for the amplification of all known isolates and clones of B19. B19 infectivity was reduced dramatically reaching approximately 3.3 and at least 5.1 log-reduction for 1 and 2 h of dry-heat treatment, respectively. The residual moisture of samples was 1.4–1.7%.

Conclusions: Dry-heat treatment completely reduced B19 infectivity to the assay limit for 2 h suggesting that the dry-heat treatment of HD is effective with the respect to possible B19 transmission.

P1654 PCR amplification in specific diagnosis of Neurobrucellosis

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Background: Brucellosis remains a health problem in Greece. Neurobrucellosis is a rare complication of the disease. Brucellosis is usually diagnosed by isolating the organism from blood culture or by a rising titer of specific antibodies in the serum. The specific diagnosis of neurobrucellosis however, presents some problems because of obscure presentation and uncommon isolation of Brucella from the cerebrospinal fluid (CSF). The aim of this study was the contribution of PCR amplification in the early diagnosis of neurobrucellosis.
Materials and methods: Six adult patients (three males, three females) who presented with Brucellosis following by neurological manifestations were assessed in this study (over a 2-year period, 2001–2002). In four of them, examination of CSF revealed an elevated cell count (10–100/mm^3), elevated protein levels (45–570 mg/dL) and low levels of glucose and in the other two examination of CSF was within normal limits. Cultures and serological examinations (SAT, CF, Coombs, B-Capt, ELISA) were performed in blood and CSF samples from all patients. PCR was performed in all CSF and sera samples. The Brucella *omp2* gene, that encodes a protein of the outer membrane, was used as DNA target (fragment 282 bp).
Results: PCR amplification produced a positive result in all CSF and sera samples from the patients with elevated results in CSF (4 patients). *Brucella melitensis* was isolated from the blood and CSF cultures in two of them, while in the other two patients only blood culture was positive for *B. melitensis*. In the two patients with normal results in CSF a positive PCR produced only in sera samples and *B. melitensis* was isolated only from blood cultures. Serological examinations in sera samples from all patients were strongly positive for Brucellosis, while in all CSF samples they were not indicative for the disease.
Conclusions: PCR is a useful test in the early diagnosis of neurobrucellosis, especially in cases with negative CSF cultures and doubtful serological tests. The assay allows distinction between the neurobrucellosis and systemic brucellosis with neurological manifestations but without actual CNS infection.

P1655 Evaluation of serodiagnostic tests in human leptospirosis: an Indian study

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Objective: Leptospirosis is an important zoonosis in Chennai, India. Serodiagnosis remains an important diagnostic tool for leptospirosis in developing countries. This study has been done to evaluate the usefulness of three serological tests.
Methods: A total number of 2053 single samples were received during January 1995–December 1996 at the Institute of Microbiology for serodiagnosis. The following tests were done: (i) MSAT (Macroscopic slide agglutination test) (ii) IgM ELISA and (iii) MAT.
Results: Current leptospirosis was confirmed in 898 samples. In 1995, of the 592 samples received, IgM ELISA was positive in 317 samples. Among these, MSAT was positive in 310 and MAT was positive in 303. In 1996, MSAT and MAT were used. Of the 1461 samples received, MSAT was positive in 581 samples and MAT was positive in 509.
Conclusions: 43.7% (898/2053) had positive serology. IgM ELISA was highly sensitive in diagnosis of current infection. MAT was useful in identifying the serovar. Autumnalis (48%) and Icterohemorrhagiae (31%) were the most common serovars detected. MSAT is a simple, quick and sensitive test which can be used as a screening test for laboratories in developing countries.

P1656 Limited genetic diversity of recent isolates of *Hemophilus influenzae* serotype *f*

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Objectives: Concern about a possible rise in the incidence of invasive infection due to *Hemophilus influenzae* serotype *f* led us to investigate the multilocus enzyme electrophoresis (MLEE) profiles of 18 recent Danish and American serotype *f* isolates and compare them with 6 old (>50 years) serotype *f* isolates and 8 recent nonserotype *f* isolates.
Methods: Biotyping and serotyping (commercial and in-house antisera) were done by conventional methods. Results of serotyping were confirmed by PCR detection of the serotype *f* specific gene. MLEE typing of 13 metabolic enzymes was determined according to the method of Selander et al. (1986).
Results: All serotype *f* strains were found to belong to biotype I. One recent serotype *f* strain did not contain the *f* specific gene sequence. MLEE typing demonstrated two genetically distinct subpopulations comprising 15 of 18 serotype *f* strains.
Conclusions: MLEE typing revealed that recent Danish and American serotype *f* strains belong to the same genetic subpopulation, distinct from older serotype *f* strains and nonserotype *f* strains.

P1657 Emergence of vancomycin intermediate *Staphylococcus aureus* in Taiwan

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Objective: One thousand and five hundred methicillin resistant *Staphylococcus aureus* (MRSA) isolates, collected from patients in the Tri-Service General Hospital in Taiwan, were screened for intermediate level of vancomycin resistance.
Methods: Brain heart infusion agar plates containing 5 mg/L vancomycin (BHIA-VA5) were used for screening of vancomycin intermediate *S. aureus* (VISA). Agar dilution method according to NCCLS and *E*-test were performed for further minimal inhibition concentration (MIC) of vancomycin. Multiplex polymerase chain reaction (PCR) and pulse field gel electrophoresis (PFGE) were also performed for genetic information of VISA.
Result: Forty-two (2.8%) of the 230 isolates that grew on BHIA-VA5 plates were confirmed to be VISA with a vancomycin MIC of 8 mg/L. The MICs results of *E*-test among the 42 isolates were lower than those tested by the NCCLS standard method. No vancomycin resistance genes, that are commonly present in enterococci, were detected in any of these 42 VISA isolates. PFGE revealed that these 42 VISA isolates belonged to 13 PFGE types, and Type A was found to be most prevalent.
Conclusion: This is the first report of the emergence and nosocomial spread of VISA in Taiwan.

P1658 Characterization of *Vibrio parahaemolyticus* isolated from patients and environment in 1995–2001

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Objectives: *Vibrio parahaemolyticus*, 148 strains, was isolated from environment (28) and diarrheal patients (120) in Korea from 1995 to 2001. The isolates were investigated on the phenotypic and genotypic characterization.
Methods: O and K serotype of isolates were determined by using *V. parahaemolyticus* antisera. Kanagawa hemolysin reaction of the isolates was tested. The antimicrobial susceptibility was studied by the disk diffusion technique. The production of thermostable direct hemoysin (TDH) determined by using reversed passive latex agglutination. The detection of *tdh*, *trh*, *gyrB*, *toxR* gene was performed by PCR method. The subspecies typing were performed by enterobacterial repetitive intergenic consensus sequence (ERIC) and pulsed-

field gel electrophoresis (PFGE). The DNA sequence of *toxRS* gene was analyzed.
Results: *V parahaemolytcis* strains, 148, were isolated from the clinical and environmental sources. In the antibiotics test, Most of isolates were resistant against ampicillin, and ticacillin and sensitive to gentamicin, kanamycin, nalidixic acid, streptomycin and tetracycline. The major O and K serotypes were O3:KUT (43%) in environmental isolates and O3:K6 (68%) in clinical isolates. All of the clinical isolates carried *toxR*, *gyrB* gene. One hundred and nine of 120 clinical isolates carried *tdh* gene and 107 of them showed Kanagawa Phenomenon. NotI PFGE differentiated 15 subtypes among the 28 environmental isolates. Twenty-five strains, selected a representative strain out of strains isolated from each outbreak, were grouped into three subtypes in analysis of PFGE and were grouped into four subtypes in ERIC PCR. In the toxRS DNA sequence analysis of 25 strains, 22 isolates were determined into 'new' group suggested by Matsumoto et al. The remaining three strains produced urease and carried *tdh* and *trh* gene.
Conclusions: Clinical and environmental isolates of *V parahaemolyticus* had similar biochemical characteristics, but almost clinical strains carried specific genes of putative virulence factor and revealed hemolytic activity. Mainly new group caused recent outbreaks of *V parahaemolyticus* in Korea. The national network and collaboration is need to investigate recent outbreaks caused by *V parahaemolyticus* in Asian countries.

P1659 Charaterization of Shiga toxin-producing *Escherichia coli* in Korea from 1998 to 2002

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Objective: In Korea, Shiga toxin-producing *E. coli* (STEC) was first isolated from a patient with brain tumor in 1998. After this case, a number of the isolates were increased gradually, 2 (1999), 3 (2000), 9 (2001) and 7 cases (2002). A total of 22 isolates was investigated on the phenotypic and genotypic characteristics and genomic DNA patterns.
Methods: STEC strains were collected through nationwide health care centers. O grouping was carried out by bacterial agglutination with antisera against 43 groups. Flagella H antigens were identified by agglutination of motile strains with 22 anti-H sera. Detection of the toxigenic genes was performed by multiplex PCR. The ability of an isolates to produce Stx1 and Stx2 was determined by a reversed passive latex agglutination test. Antimicrobial susceptibility of the isolates was studied by the disk diffusion technique. The subtypes were determined with the pulse field gel electrophoresis (PFGE).
Result: All of STEC strains were isolated from sporadic cases except small outbreak involved two patients infected with serotype O171. The distribution of O serotypes was O157 (5), O111 (4), O26 (4), O171 (2), O128 (1), O91 (1), O55 (1) and OUT (8). Ten of the 22 STEC isolates carried *stx2* gene only, 6 carried the *stx1* gene only and 6 carried both genes. The corresponding toxins were produced by 21 of 22 strains. The one isolate, which did not produce Stx, was O128:H4. The *eaeA* and *hlyA* genes were detected in all isolates except 3 and 4 strains, respectively. The identical PFGE patterns were not shown even among the same O serotype group except O171 serotype involved outbreak.
Conclusion: There is not yet big outbreak caused by STEC infection in Korea. But the incidence of infection is gradually increasing. Korea is located close to Japan, where there are big outbreaks every year, and heavy materials and travelers come and go to each country. National Institute of Health forced local health care center to survey actively STEC.

P1660 Studies on detection and type identification of *Orientia tsutsugamushi* in a single larval mite of *L. scutellare* by polymerase chain reaction

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Objective: To detect and type the *Orientia tsutsugamushi* DNA in a single larva of 61 *Leptotrombidium scutellare* collected from the endemic areas of tsutsugamushi disease in Jiangsu province in China.
Methods: The group-specific primers and the type-specific primers designed according to the nucleotides sequence of the gene region encoding the 56 kDa antigen of Ot were used to detect and type the Ot by polymerase chain reaction.
Result: Ot specific DNA band of about 320 bp were observed in two of the 61 larval mites after PCR amplification using the group-specific primers for detection, showing that the two larvae carried Ot and the Ot. Carrying rate of these mites was 3.7%. Identifying the DNA using the type-specific primer resulted that the two Ot belonged to a new serotype and dominant strain in epidemic region in Jiangsu and they were similar to Kawasaki strain of Japan and the homology of their nucleotide sequence was 96.8%.
Conclusion: This method proved to be applied in detecting the Ot in a single mite larva, which is useful for the epidemiology analysis of the transmitter in endemic areas of scrub typhus.

P1661 Expression and identification of truncated 56 kDa protein of *Orientia tsutsugamushi Gilliam* strain in prokaryotic cells

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Objective: The purpose of this studies is to express the truncated 56 kDa gene of *Orientia tsutsugamushi* (Ot) *Gilliam* strain in *Escherichia coli* to obtain the abundant recombinant protein with high activity and to play a foundation for preparing the diagnosis kit of scrub typhus.
Methods: The truncated gene (about 700 bp) encoding for the C-terminus amino acids of 56 kDa protein of Ot with high hydrophilicity and hemologization was amplified by using PCR technique. PCR product was cloned initially into the PGEX-4T-2 expression vector, then transformed into *E. coli* TGI, the plasmid DNA was extracted and digested with enzymes. Plasmids containing the right insertion were sequenced to confirm its identity and retransformed the combinants into *E. coli* BL21 (DE3). After IPTG induction, the bacterial lysates were analyzed by SDS–PAGE and Western blot.
Results: The recombinant plasmid containing the aim gene has been expressed successfully, the expressed fusion protein was visualized on gel at molecular mass about 58 kDa and can be detected by the positive serum from patients of scrub typhus by Western blot.
Conclusions: The expression product of the truncated 56 kDa gene of *Gilliam* strain has immunoreactivity and may be a promising diagnostic antigen for further preparing the diagnosis kit of scrub typhus.

P1662 Resuscitation and mouse virulence of viable nonculturable halophilic noncholera vibrios

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Objectives: When enteric and pathogenic bacteria are released from their hosts into natural environments, they are challenged by various environmental stresses. These bacteria may persist outside of the host by entering a viable nonculturable state (VBNC): cells appear dormant and cannot be cultured, but are able to return to the active metabolizing state. The VBNC state has been found in numerous human pathogens, including *Vibrio* species. Among halophilic noncholera vibrios, *Vibrio vulnificus* and *Vibrio alginolyticus* are able to entry in the VBNC state. Whether or not *V alginolyticus* and *V vulnificus* remain virulent during, entry into and resuscitation from the VBNC state has not been definitively demonstrated.
Methods: *V vulnificus* and *V alginolyticus* obtained from fresh and frozen seafood were used. The microcosm water method described by Oliver and Bockian (Appl. Environ. Microbiol. 1995. 61: 2620–2623) was used to obtain nonculturable cells. The culturability of cells was determined by spread plate counting on HI agar. To examine both entry to and resuscitation from the VBNC state in natural environment, we employed the membrane diffusion chambers described by McFeters and Stuart (Appl. Environ. Microbiol. 1972. 24: 805–811) and Oliver et al. (Appl. Environ. Microbiol. 1995. 65: 2624–2630). Virulence assay was performed as described by Ottaviani et al. (Lett. Appl. Microbiol. 2001. 33 : 61–64) and by Oliver and Bockian (Appl. Environ. Microbiol. 1995. 61: 2620–2623).
Results: The entry of *V alginolyticus* and *V vulnificus* into VBNC state was induced by incubation in 8–15°C water with salinity of 12–15% to obtain a population <0.1 cfu/mL following plating on HI agar within 12 days. Virulence was determinated using a iron-overload mouse model. Mice were injected at various time with PBS microcosms before and after cells become nonculturable and LD50 were calculated. When mice dead as a result of challenge of PBS containing VBNC cells, culturable cells of the same strain were isolated from both the peritoneal cavity and blood samples taken from

the dead mice. These data were observed in *V. vulnificus* and *V. alginolyticus* strains showing mouse virulence.
Conclusions: The possibility that *V. alginolitycus* and *V. vulnificus* cells might enter into a dormant state undetectable by routine plating methods presents an important epidemiologic problem, especially if bacterial cells are able to retain virulence during starvation.

P1663 Nanobacteria antigen and antibody titers in USA controls compared with UK controls and kidney disease patients

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Background: Nanobacteria (Nb) are newly discovered microbial agents that are unique due to small size (0.2–0.5 nm) and a calcium apatite shell. Nb are hypothesized to be the cause of extra-skeletal calcifications, e.g. kidney stones, atherosclerotic lesions and renal cysts. Nb associated biogenic apatite is both antigenic and immunogenic; calcium apatite is not.
Objectives: In this initial survey, sera from 22 patients with various chronic renal diseases (UK-P) and controls (blood donors) from the UK (20; UK-C) and USA (20; USA-C) were tested for antibody (Ab) and antigen (Ag) to Nb.
Methods: ELISA kits were used (Nanobac Oy, Kuopio, FI). For this study, unit values of Ab and/or Ag greater or equal to 1.0 were considered positive. Samples for Ab were diluted 1 : 500; for Ag, 1 : 5 and 1 : 10 due to prozone-like effect observed at recommended 1 : 1 dilution.
Results: Nb Ab positivity was >2.5-fold (35%) than USA-C (14%); UK-C positivity was >2-fold than renal patients (18%). Nb Ag positivity was similar for the three groups (mean 63%; range 59–69). Ag/Ab titers were examined for various permutations of positivity (see Table 1).

Table 1 Comparative Ab and Ag results for USA and UK individuals

Ab	Ag	UK-C (%)	USA-C (%)	UK-P (%)
+	+	20	5	9
+	−	15	9	9
−	+	40	64	50
−	−	25	22	32

Nb Ab or Ag positivity did not correlate with age (range: 23–68 years) or gender.
Conclusions: This initial study for Nb Ab and Ag in presumed healthy individuals in Scotland and Central Illinois revealed a >2-fold difference in positivity. The relationship between Nb positivity and acute/chronic human disease(s) involving soft tissue calcification is unknown. Interestingly, Scotland has a higher rate of cardiovascular (i.e. calcified plaque) disease than USA. Kidney patients had lower Nb positivity perhaps due to being on hemodialysis, thus clearing Nb more effectively. Expanded studies are warranted to determine precise breakpoints for Ag/Ab positivity and negativity in health and various disease states.

PCR and viruses

P1664 Evaluation of a new quantitative CMV PCR assay in allogenic stem-cell transplant patients

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Purpose: To evaluate a new commercial available quantitative PCR assay (affigene® CMV VL Test) (PCR-AVL), in allogeneic patients (ASCT), by comparing the results obtained with the new PCR-AVL with those obtained with Cobas Amplicor CMV Monitor™ assay (PCR-CA) and the pp65 antigenemia (Ag) assays.
Methods: A total of 100 plasma samples from 5 mieloablative allogenic stem-cell transplant (SCT) patients (from HLA-identical siblings) with a total of seven CMV episodes were included (15–28 samples per patient). Only one patient developed a nonfatal CMV disease. Ag was prospectively performed following standard procedures and was used for patient management. Samples for PCR were frozen at −80°C and tested afterwards. PCRs were performed following manufacturer recommendations. Ag was considered positive when at least two positive cells were seen counting two slides ($\geq 2+$ cells/400 000). PCR-CA were considered positive when $\geq$400 DNA-copies/mL and PCR-AVL when $\geq$1 DNA-copies/mL.
Results: Fifty-seven samples (57%) yielded a positive CMV result in at least one assay: 53/99 (53.5%) with PCR-AVL, 46/99 (46.5%) with PCR-CA and 34/96 (35.4%) with Ag. One sample was excluded due to invalid result by both PCR assays. Twenty-nine samples were positives by the three methods and 40 were negative by all three assays. There was a high correlation between PCR-AVL assay vs. the PCR-CA ($r = 0.89$). The median number of copies detected by PCR-AVL was lower than PCR-CA. The $\log_{10}$ differences between the PCR-AVL and the PCR-CA assay were plotted against the average $\log_{10}$ CMV viral load/mL resulting in up to 1.8 log lower result with the PCR-AVL assay compared with the PCR-CA. CMV infection was detected earlier (6–11 days) in four out of seven episodes by PCR-AVL and PCR-CA assays than by Ag. Moreover, in 1 episode, PCR-AVL detected infection 7 days earlier than PCR-CA and Ag. The capability of the PCR-AVL to monitor the viral load in patients during the follow-up was evaluated by plotting post-transplant day against CMV viral load/mL for the PCR-AL and PCR-CA assay and Ag. The PCR-AVL was capable of monitoring both increase and decreases in the CMV viral load with same efficacy as the PCR-CA and the Ag assay.
Conclusions: The PCR-AVL assay showed a good correlation with the PCR-CA and the Ag assays. The PCR-AVL assay is also capable of monitoring increases and decreases in the CMV viral load. This quantitative CMV PCR seems a useful tool in CMV monitoring after SCT.

P1665 Evaluation of real-time PCR-based assays for the detection of herpesvirus and enterovirus in CSF

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Objectives: PCR is recognized as a reference standard method for the rapid, sensitive and specific detection of enterovirus and herpesvirus in cerebrospinal fluid (CSF). In the present study we report the analytical evaluation of real-time PCR-based assays and a one year experience in the routine diagnosis of enterovirus and herpesvirus of the central nervous system.
Methods: Quality Control for Molecular Diagnostics (QCMD) proficiency panels for Enterovirus, HSV, CMV and UK NEQAS for the Molecular Detection of Viruses in CSF, long with serial dilutions of positive clinical samples for EBV and HHV-6 were used to assess the sensitivity and specificity of the assays. Enterovirus RNA was amplified by an 'in-house' developed nested RT-real-time PCR in the SmartCycler (Cepheid, USA) using a TaqMan probe. HSV-1, HSV-2, CMV and HHV-6 genomes were amplified with sets of primers and eclipse probes from Epoch (USA) with Roche's Fast-Start-Taq DNA Polymerase in the SmartCycler; VZV and EBV were amplified with kits from Artus (Germany) in the LightCyclerâ (Roche, Germany). These assays were further applied to a total of 279 CSF samples collected, during 2002, from patients with clinically suspected viral infection of the CNS.
Results: All enterovirus containing samples from the proficiency panels tested positive including the most diluted sample (0.036 TCID 50/mL); the specificity observed was 100%. Thirty-two percent of the 209 CSF samples were found positive, a frequency similar to that observed in 2001 (31%) by conventional nested RT-PCR. The detection limit (copies/mL) of the Herpesvirus assays was found to be 200 for HSV1, 100 for HSV-2, 500 for VZV and 315 for CMV. HHV-6 was detected up to a 1/50 dilution of a positive sample, whereas EBV was detected up to a 1/10 dilution. All negative samples from the proficiency panels were found negative (100% specificity). Of the 161 CSF samples tested for herpesvirus, 16 (10%) were found positive (2 HSV-1, 2 HSV-2, 2 EBV, 5 VZV and 5 HHV-6).
Conclusions: The use of real-time PCR methodology has allowed a dramatic decrease in the turn-around time: from 48 h for enterovirus and 36 h for herpesvirus to 4 h in both cases. The specificity of the assays was maintained at

100% in all cases, whereas the sensitivities were found to be similar or better (CMV) to the conventional PCR assays. In conclusion, the use of real-time PCR allows a faster response time, without sacrificing either sensitivity or specificity.

P1666 Recovery of HIV-1 RNA from plasma using magnetic silica particles used for viral load determination and antiviral drug resistance DNA chip tests

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Objective: A second-generation manual extraction methodology with increased sample throughput and user convenience is under development at bioMérieux. The objective of this study was to investigate the suitability of the new method for the recovery of HIV-1 RNA from plasma samples in combination with (i) viral load measurement and (ii) antiviral drug resistance mutations detection using real-time NASBA and DNA Chip technology, respectively.
Methods: HIV-1 RNA was extracted from human plasma samples using magnetic silica particles. Briefly, 1 mL of plasma was added to 2 mL lysis buffer. Subsequently the released HIV-1 RNA was bound to magnetic silica particles and washed several times using different buffers. The RNA was recovered from the silica particles by applying heat and a dedicated elution buffer. The value of this method as a front end for viral load applications was measured by testing 168 samples that were spiked with different concentrations HIV-1 RNA and 18 EDTA plasma samples obtained from HIV-1 infected patients in combination with the bioMérieux NucliSens EasyQ HIV-1 assay. As a reference isolation method, the NucliSens Extractor was used. For the DNA chip study, nucleic acid from 43 plasma samples were extracted and analyzed for mutations in a subset of HIV genes by a new HIV-1 DNA Chip (designed by BioMérieux and manufactured by Affymetrix).
Results: The analytical sensitivity in combination with NucliSens EasyQ HIV-1 was 45 IU/mL for the new extraction method, which is comparable to the analytical sensitivity as measured in combination with the NucliSens Extractor (62 IU/mL). In the 18 clinical samples that were used for the viral load study, a good correlation in quantification result was found compared with the known viral load data ($y = 0.96x + 0.25$ with $r^2 = 0.97$). The 43 samples analyzed by DNA chip showed a good correlation compared with sequence analysis. The correct identification of key resistance mutations for the protease and the whole reverse transcriptase was 97% and 94%, respectively. No resistance mutation in the integrase and gp41 were found, mutations in the gag cleavage sites (A431V or Q430R) were measured in 21% of the patients.
Conclusions: The new nucleic acid extraction method was successfully used to recover HIV-1 RNA from plasma samples in combination with NucliSens EasyQ HIV-1 viral load measurements and for HIV-1 antiviral drug resistance mutations detection using the DNA Chip technology.

P1667 Determination of human T-cell leukemia virus proviral load by a multiplex one-tube real-time PCR quantitation assay

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Objectives: Current diagnostics of the human T-cell leukemia virus types 1 and 2 (HTLV-1/−2) is mainly based on antibody screening using ELISA and Western blotting (WB). The majority of these assays allow for neither discrimination between the virus types nor for quantitation of proviral load. Therefore, the aim of this study was to develop a rapid and highly sensitive HTLV diagnostic tool based on real-time PCR which allows both a determination of proviral load and a differentiation between the virus types. To warrant a high reliability of the assay and to rule out false negative results, an internal control was to be included.
Methods: For the design of a real-time PCR based diagnostic assay primers and fluorescence labeled probes specific for a conserved sequence in the proviral Tax region of the HTLV genomes were designed. As an internal control a second heterologous amplification system of the house-keeping gene beta-globin was included. The assay was established on three real-time instruments (LightCycler®, Rotor-Gene, TaqMan). For quantification purposes, a serial dilution of a plasmid mixture containing the HTLV and beta-globin amplicon was prepared.
Results: The sensitivity of this real-time PCR assay (RealArt™ HTLV PCR Reagents) was down to 1 copy/PCR on the LightCycler® instrument for both HTLV-1 and -2 and for the beta-globin gene. Modified HTLV detection systems were established on the Rotor-Gene and the TaqMan real-time PCR instruments and exhibited a comparably high sensitivity performance. The beta-globin amplification system allowed the determination of the proviral load per genome equivalents and serves as an internal control. A melting curve analysis allowed an unequivocal discrimination between the two HTLV types. The real-time PCR assay was verified using clinical specimens of varying concentrations.
Conclusions: The new real-time PCR assay provides a cost-and-time effective diagnostic test for the quantitative detection of HTLV-1 and -2. The high sensitivity of the analytical and the internal control PCR exceeds that of many serological assays and allows a precise determination of the proviral load. In addition, the internal control amplification indicates an effective DNA preparation and the absence of PCR inhibitors. In summary, this new RealArt™ system provides a versatile tool for both routine blood donor screening and patient treatment success assessment.

P1668 Evaluation of reverse transcription polymerase chain reaction for detection of tick-borne encephalitis virus genome

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Objectives: Tick-born encephalitis virus (TBEv) is endemic in many Eastern and Central European countries. This pathogen is considered as an important etiological agent of viral neuroinfection in Poland with 150–200 of TBE cases reported annually. The laboratory diagnosis of acute TBE infection based on detection of TBEv-specific IgM antibodies in serum sample and estimation of local antibody synthesis by the examination of serum IgG/CSF IgG ratio. Isolation of TBEv relies on intracerebral inoculation of mice and observations symptoms of infection. Application of nucleic acid amplification techniques has become more and more popular in diagnosis of neuroinfection. Our aim was examination of RT-PCR method for diagnosis of TBE based on study performed on 25 CSF samples selected from subjects with serologically confirmed acute TBEv neuroinfection. In addition, employment of RT-PCR method for detection of TBEv genome in brain of TBEv infected mice was investigated. TBE virus propagated in Vero cells and inactivated virus from TBE vaccine were used as a control of TBEv RT-PCR specificity.
Methods: Serological designations of serum and CSF samples were performed using ELISA IgG/IgM assay (PROGEN BIOTECHNIK). RNA was extracted from CSF sample using QIAamp Viral RNA Mini Kit (Quiagen) according to kit directions or using phenol–chloroform extraction in a case of brain samples. RT-PCR was performed in a two-step process. Primer sets (Gibco-BRL) in TBE amplification system were directed to 5′NCR region of TBE virus genome and gave a product of amplification of 128 bp in size.
Results: Positive results of TBEv specific RT-PCR were obtained with propagated in Vero cells TBE virus as well as in brain of infected mice. Genome of TBE virus was also detected in inactivated TBE vaccine. But from a total of 25 cerebrospinal fluids samples from patients with serologically confirmed TBE, only in one subject result of TBEv specific RT-PCR was positive.
Conclusions: Typically biphasic course of tick-born encephalitis virus infection effects that serological designation is more convenient in laboratory diagnosis of TBE encephalitis than usage of nucleic acid amplification method. Conversely, amplification techniques are very effective for detection TBE genome in brain and other biological materials.

P1669 Comparison of two commercial assays for hepatitis B virus DNA quantification: single vs. double testing

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Objectives: To compare the performances of Digene Hybrid Capture (DHC) and Bayer Quantiplex (BQ) HBV DNA assays with regard to precision of the single vs. double testing of the samples and to determine the conversion between the two different assay values.
Methods: Intra- and interassay variabilities were tested in five runs by five patient sera containing HBV DNA over the range of 5–2000 pg/mL. Thirty-six additional sera with the same range of HBV DNA were tested in duplicate

by the two commercial assays and coefficients of variation (CV) were determined. Correlation between HBV DNA results of DHC and BQ was examined by regression analysis.
Results: The mean intra- and interassay CV for the DHC and BQ assays were 11.6% to 9.3% and 19.3% to 19.2%, respectively. Among the duplicate studied sera, 3(9.7%) and 7(19.3%) samples required repeat testing in the DHC and BQ assays, respectively, as their CV exceeded the limit of 30% ($P > 0.05$) A good linear relationship was observed between the logarithmic conversions of HBV DNA levels determined by the two assays ($r^2 = 0.969$, slope = 1.037). The formula for the conversion of results was log(HBV DNA level by BQ) = 0.934 × log(HBV DNA level by DHC) + 0.372. The difference between the $\log_{10}$ HBV DNA levels of the two different assays on the same sample was always <0.5.
Conclusions: The $\log_{10}$ results of the two assays were closely correlated for the sera containing HBV DNA within the detection range of both assays. The difference for the same sample was always <0.5 $\log_{10}$. Sera with an unacceptably high within-run CV value were detected in both assays, the number was higher by BQ but the difference was not significant. Single instead of double testing of sera by BQ may cause inaccurate results in some settings.

P1670 Detection of herpes simplex virus DNA and enterovirus RNA in cerebrospinal fluid using PCR and microplate or strip hybridization assay

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Objectives: To compare the microplate and strip hybridization assays for the detection of herpes simplex virus (HSV) DNA and enterovirus (EV) RNA PCR products in cerebrospinal fluid (CSF) samples.
Methods: Thirty-one CSF samples from patients with a clinical diagnosis of aseptic meningitis were studied. Nucleic acid was extracted from 200 µL of CSF using High Pure Viral Nucleic Acid Kit (Roche Applied Science). Primers from the DNA polymerase gene were used for HSV PCR. EV RT-PCR was performed in a two-step process using primers from the 5′ noncoding region. PCR products were labeled with digoxigenin by using PCR ELISA Dig-Labeling kit (Roche Applied Science). Detection was done by gel electrophoresis and by biotinylated probe using streptavidin-coated microplate and streptavidin-coated strips (Roche Applied Science).
Results: Among the 31 CSF samples, three (9.7%) were HSV PCR and 12 (38.7%) were EV RT-PCR positive by gel electrophoresis, microplate and strip hybridization. Two samples (one HSV and one EV) were only detected by microplate assay with a low OD value (<600).
Conclusion: Strip hybridization assay might be an alternative to the gel electrophoresis with an advantage of using sequence specific probe. It can be used for a single specimen and takes shorter time than microplate detection but is not as sensitive.

P1671 Qualitative and quantitative determination of herpes virus nucleic acid in clinical samples

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Objectives: The aim of this study was to compare real-time quantitative PCR with multiplex nested PCR for the detection of the herpes viruses CMV, HSV-1, HSV-2, VZV, EBV, and HHV-6 in clinical samples and to determine the viremias of the positive ones.
Materials and methods: A total of 353 clinical samples and performance controls were tested by real-time quantitative PCR on the LightCycler instrument and by multiplex nested-PCR on a conventional thermocycler (140 samples of brain or CSF (group 1), 55 urogenital swabs (group 2), 22 biopsies from the gastrointestinal tract (group 3), 24 urine samples (group 4), 58 blood samples (group 5), 15 specimens from the respiratory tract (group 6), 12 samples from various organs (group 7), and 16 specimens from external quality control panels (group 8)).
Results: A total of 325 (92.1%) out of 353 samples gave the same qualitative result in both methods and only these are included in the following evaluation. CMV, HSV-1, HSV-2, EBV, VZV and HHV-6 were found 1, 8, 2, 9, 6, and 3 times in 132 samples from group 1. The corresponding numbers were 0, 14, 15, 2, 2, and 0 for 55 samples from group 2; 3, 3, 0, 6, 0, and 5 for 22 group 3 specimens; 12, 0, 0, 0, 0, and 0 for 24 urine samples; 17, 3, 0, 15, 1, and 2 for 58 blood samples; 2, 5, 0, 4, 0, and 0 for 9 group 6 samples; 3, 0, 0, 4, 0, and 0 for 10 samples from various organs, and 0, 5, 5, 0, 2, and 0 for 15 external quality control samples, respectively. DNA copy numbers of CMV were low in all kinds of positive specimens (median ≤ 500–16.500/mL). HSV-1 DNA was highest in urogenital swabs (median = 33.575.000/mL) followed by brain/CSF (median = 82.130/mL), respiratory samples (median = 8.800/mL), blood (median = 459/mL), and biopsies from the gastrointestinal tract (median = 93/mL). HSV-2 was most prevalent in urogenital swabs followed by brain/CSF and blood, VZV and EBV-DNA were also found highest in urogenital swabs. HHV-6 positive samples of brain/CSF, GI-tract and blood contained nearly equal median copy numbers.
Conclusions: Both PCR methods seem to be equally suited for the detection of herpes virus DNA in clinical samples as 92.1% yielded equal results. Quantitative values of HSV1-, HSV2-, EBV- and VZV-DNA were extremely high in urogenital swabs. Median titers of CMV, HSV-1, HSV-2, VZV, EBV, and HHV-6 in clinical samples other than urogenital swabs seem to be low.

Treatment of hepatitis band C

P1672 Evaluation of response and safety of chronic HBV infection treatment with alpha-INF or combined treatment with alpha-IFN and lamivudine

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Objective: Effectiveness and safety evaluation of treating HBV infection with either alpha-IFN or combined treatment with alpha-IFN and lamivudine.
Methodology: Eighteen chronic HBV infection patients have been studied, 13 men and 5 women, average age 44.1, increased transaminase level of 1.5 above normal, HBV-DNA (+). Three out of 18 HBeAg (+), 15/18 HBeAg (−). Biochemical response study: 4 months. Virology response study: 6 months. Histology response study: 12 months. All studies conducted after treatment end.
Results:
1. 3 HBeAg (+) patients underwent alpha-IFN monotherapy. Two showed biochemical response and one showed virological response. One patient stopped treatment due to praecox side-effects. Improvement of histological findings was diagnosed in none.
2. 15 HBeAg (−) patients underwent alpha-INF treatment, eight showed biochemical response, seven showed virological response. three interrupted treatment due to side-effects. After treatment end, two patients had praecox relapse, with increased ALT and PCR (+) levels. In general, five responded to treatment, and one was found with long-term remission (SVR) Improvement of histological condition was found in two patients (improvement of infection and fibrosis reduction).
3. 15 HBeAg (−) patients, four underwent combined treatment with alpha-IFN and lamivudine. Three showed biochemical response and four virological response. None interrupted treatment due to side-effects. All patients responded to the treatment. One patient was found with histological condition improvement.

Conclusions: Combined treatment with alpha-INF and lamivudine administered to HBeAg (−) patients, brings better results compared with alpha-IFN monotherapy. It seems to be equally safe to monotherapy, but it should be clearly stated that more cases need to be studied in order to firmly establish our conclusions.

P1673 YMDD mutations during lamivudine therapy in patients with chronic hepatitis B

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Objectives: The emergence of a YMDD mutant resistant to lamivudine therapy has been reported in patients with chronic hepatitis B treated with lamivudine therapy. The aim of this study was to evaluate the development the YMDD mutant during lamivudine therapy.
Methods: We investigated the emergence of the YMMD mutant by reverse hybridization line probe assay (INNO-LIPA HBV DR) in patients with chronic hepatitis B who were treated with lamivudine for 12.8 ± 3.8 months.
Results: A total of 29 biopsy proven chronic hepatitis B patients who received lamivudine 100 mg daily were included the study. Twenty-two cases were male and seven were females with their ages averaging 23.7 ± 8.6 (15–45 years). Family history of hepatitis was positive in 51.7%. Pretreatment levels of AST, ALT and HBV DNA were 63 ± 30, 89 ± 64 (U/L), 2742 ± 2315, respectively. Mutations in the YMDD motif of the DNA polymerase gene were identified in seven patients (24.1%) in the 11.28 ± 2.6 month (6–15 month) of lamivudine treatment. The mean levels of HBV DNA in cases with and without YMDD mutation were 4329 ± 2636 and 2237 ± 2013, respectively ($P = 0.02$).
Conclusions: It is determined that YMDD development is affected by the high levels of HBV DNA in patients with chronic hepatitis B. This finding demands new therapeutic approaches such that a tailor-made, individualized treatment regimen allowing a better insight into the variability in response.

P1674 Affigene® HBV patient disease management concept-tools for individualizing HBV treatment

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Background: The outcome of treatment of chronic HBV is dependent on several different factors, e.g. the level of HBV DNA, and the heterogeneity of the viral strain (e.g. drug resistant viruses and precore mutants). This indicates that individualization of treatment of HBV based on these parameters would be beneficial for the patient, giving increased well-being as well as from a health economic perspective. The affigene HBV patient disease management (PDM) concept offers a panel of assays for HBV monitoring: (i) a quantitative HBV-DNA assay; affigene HBV VL (ii) a semiquantitative precore/core mutant assay; affigene HBV mutant VL and (iii) a qualitative lamivudine resistant assay; affigene HBV DE/3TC. This poster describes the performance of the three assays and the assessment on clinical material.
Methods: Each assay is based on four major processes. (i) specimen preparation (ii) PCR amplification of target DNA using HBV specific primers (iii) hybridization of target specific probe or elongation of a mini sequencing primer (iv) colorimetric detection. Affigene HBV mutant VL and affigene HBV DE/3TC are both based on the mini sequencing technique while the affigene HBV VL assay is based on a DNA hybridization method. Performance studies were evaluated on standardized WHO HBV samples or artificial specimens comprised of DNA fragment mixes of different mutant genotypes. The clinical samples were from patients with chronic HBV infection. Each clinical sample was prepared once using the affigene HBV Specimen Preparation kit and analyzed with all three assays. The results were compared with Amplicor HBV Monitor or sequencing.
Results: Performance data on the affigene HBV PDM concept is shown in Table 1. The viral load is expressed in international units per ml (IU/mL). Excellent correlation ($r = 0.99$) was obtained when comparing the affigene HBV VL and the Amplicor HBV Monitor assays. Both affigene HBV mutant VL and HBV DE/3TC showed a good agreement to sequencing. However, data indicate that the affigene HBV mutant VL and DE/3TC are more sensitive in detecting a mixed population of wild-type and mutants.

Table 1 Performance data on affigene HBV PDM concept

	Dynamic range (IU/ml)	Limit of detection (LOD) (IU/mL)	Sensitivity at LOD (% mutant)
Affigene HBV VL	up to 8×10^6	100	n/a
Affigene HBV Mutant VL	500–8×10^6	5000	5
Affigene HBV DE/3TC	15000–8×10^6	15000	15

Conclusions: The affigene HBV PDM concept offers an extensive view of the HBV DNA status. The three standardized assays for monitoring patients with chronic HBV provide guidance for individualized treatment of the infection.

P1675 Predictive value of serum B2-microglobulin (B2-m) levels for virological breakthrough, in chronic hepatitis B patients under long-term lamivudine treatment

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Objectives: To evaluate the predictive role of serum B-m levels in virological breakthrough (VB) among HbeAg (–) chronic Hepatitis B (CHB) patients under long-term lamivudine monotherapy.
Methods: Serum samples were obtained at baseline and every 3 months during treatment, from 25 CHB patients, serologically, biochemically, virologically and histologically confirmed. Serum HBV-DNA was calculated using a PCR assay (Amplicor, Roche) and serum B-m levels using a microparticle enzyme linked immunoassay (AxSyM, Abbott). All CHB patients were naïve and received LAM monotherapy for 36 months. Statistical evaluation was done using ANOVA *t*-test and Cox-regression analysis (*P*-value <0.05).
Results: Seven out of 25 (28%), 10 out of 25 (40%) and 13 out of 25 (52%) CHB patients exhibited VB at month 12, 24 and 36, respectively. All CHB patients were HBV-DNA (–) at month 6 of treatment. Baseline serum HBV-DNA and serum B-m levels were comparable between the responders group (RG) and VB. Eighteen CHB patients exhibited a significant elevation of serum B-m levels at month 3 and 6 of treatment (11 RG/7 VB) and 7 CHB patients exhibited decline (1 RG/6 VB). Baseline HBV-DNA was significantly lower in CHB patients who exhibited elevation of serum B-m levels than in those who exhibited decline ($P < 0001$). The duration (in months) of B2-m elevation was significantly higher in the RG than VB (6.6 ± 1.89 vs. 3.5 ± 3.5, $P = 0024$).
Conclusion: Elevation of serum B2-m levels, especially during the first months of LAM monotherapy, was observed in the majority of CHB patients with low baseline viremia and was correlated with long-term virological response, suggesting an activation of endogenous immune response, following the reduction of viral load, in this group of patients.

P1676 The effect of lamivudine therapy in chronic hepatitis B patients

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Objectives: Lamivudine is an oral nucleoside analogue with a potent antiviral effect on HBV due to inhibition of viral DNA polymerase. In this study response to treatment with lamivudine in Iranian patients was evaluated.
Methods: Nineteen patients were studied. Lamivudine 100 mg daily per oral for one year was prescribed. Before the beginning of the treatment, virus markers (HBSAg, HBV DNA, HBeAg) and liver enzymes were recorded and liver biopsy were taken. These experiments were repeated after treatment too. and the results were recorded.
Results: Among the 19 patients under study 12 (63.19%), 14 (73.6%) and 11 (60%) had virological, biochemical response and histological improvement, respectively.
Conclusion: Several nucleoside analogues active against HBV are being evaluated. Famciclovir and ganciclovir have only limited activity against hepatitis B. However, lamivudine which inhibits reverse transcriptase activity of both HIV and HBV, is a potent and effective agent for patients with chronic hepatitis B.

P1677 Clinical side-effects in patients with chronic viral hepatitis C either of antiviral treatment with alpha-IFN or combined treatment with ribavirin, or combined treatment with peg-intron E ribavirin

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Introduction: Evaluation of standardized initial treatment of chronic hepatitis C with recombinant alpha-IFN or in combined treatment with Ribavirin, or in combined treatment with Peg-Intron and Ribavirin (although this treatment causes numerous side-effects).
Methodology: For the treatment of chronic hepatitis C, we used alpha-IFN at a dose of 3 MU 3 times a week for a period of six to 12 months, or combined treatment with Ribavirin. Fifteen mg/kg for 6 months, or combined treatment with Peg-Intron 100 μg per week and Ribavirin 15 mg/kg to 51 patients (39 male patients, 12 female ones, average age 42.3-years-old).
Results: The side-effects should be divided as early ones (taking place within the first two weeks) and postponent ones (occurring after two weeks). Early side-effects include anemia (14/51 patients), leukocytopenia (26/51), thrombocytopenia (18/51), myalgia (6/51), influenza phenomena (6/51), and fever (7/51). Postponent side-effects include weight-loss (14/51), defluation (14/51), libido decrease (8/51), insomnia (12/51), headache (5/51), abdominal pain (4/51), diarrhea (2/51), stress (7/51), itching (3/51), weakness (7/51), depression (6/51), rash (1/51), dizziness (3/51), xerostomia (1/51), xerodermia (3/51), anorexia (10/51), chills (sense of cold 2/51), surdity (1/51), arrhythmia (1/51), S. Raynaud (1/51) and fibular nerve paresis (1/51). No patient mentioned taste disorders. Six patients had to stop treatment because of the side-effect symptoms' severity.
Conclusions: The treatment of chronic hepatitis C with either alpha-IFN, or combined treatment with Ribavirin, or combined treatment with Ribavirin and Peg-Intron appears to be connected with a variety of side-effects, although these side-effects rarely lead to the modification or interruption of the treatment.

P1678 Evaluation of chronic HCV infection response and safety with alpha-IFN or alpha-IFN and ribavirin combined treatment or peg-intron and ribavirin combined treatment

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Objective: Evaluation of chronic HCV infection response and safety with alpha-IFN or alpha-IFN and ribavirin, or peg-intron and ribavirin combined treatments.
Methodology: Fifty-one patients were studied. Average age 42.3, transaminase increase 1.5 above normal and HBV-RNA (+). Biochemical response studied for 4 months, virological response for 6 months and histological response for 12 months after the end of treatment.
Results: Alpha-IFN Monotherapy: 13 patients, 8 men, 5 women/average age 38.7. Biochemical response: 10; Virological response: 8 patients. Long-term virological response (SVR): five patients/HCV RNA non detectable by PCR method. two patients stopped treatment due to side-effects. Praecox relapse after treatment end: one patient. Histological examination in two patients one year after treatment end, showed infection reduction and same degree of fibrosis. Alpha-IFN and ribavirin combined treatment: 29 patients, 22 men, 7 women/average age 37.3; Biochemical response: 26 patients; Virological response: 22 patients. Long-term virological response (SVR): 13 patients/HCV RNA non detectable by PCR method. Two patients stopped treatment due to side-effects. Praecox relapse after treatment end: one patient. Histological examination in nine patients, showed infection and fibrosis degree reduction, while fibrosis increase was found in one patient. Peg-intron and ribavirin combined treatment: nine patients, seven men, two women/average age 40.3. Biochemical response: seven patients; Virological response: seven patients; two patients stopped treatment.
Conclusions: Alpha-IFN and ribavirin or peg-intron and ribavirin combined treatments produce better results than a-IFN monotherapy. Combined treatments are equally safe to monotherapy and the effectiveness of combined treatments is related to genetypes 2 and 3, the young age of patients and the initial mildness of the fibrosis.

P1679 Sustained virological response in patients with chronic hepatitis C virus viremia treated with ribavirin (RIB) alone followed by interferon alpha and RIB combined

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Objectives: Combined interferon (IFN) and RIB therapy is widely accepted for Japanese patients with chronic hepatitis C virus (HCV) viremia. RIB has been shown to significantly increase the virological response to IFN. Although RIB alone doesn't have a substantial impact on HCV clearance, it has been shown to normalize alanine aminotransferase (ALT). The above suggests that RIB may modulate the immune system against HCV. The aim of this study was to investigate the possible relationship between virological response and expansion of T helper (Th) 1 cells by sequential administration of IFN and RIB combined after 4 weeks of RIB administration.
Methods: Forty consecutive patients with HCV of genotype 1b and with a high HCV RNA level, over 100 KIU/mL, were treated with RIB for 4 weeks, after which they received a combined IFN-alpha and RIB treatment for 24 weeks. Changes of serum ALT and the HCV RNA level of each patient was measured during the observation. Virological response was defined as negative for HCV RNA in serum. We also used flow cytometry to investigate sequential changes of IFN-gamma producing (Th1) and interleukin-4 producing (Th2) cells from whole blood samples after stimulation with PMA and ionomycin.
Results: During the 4-week RIB administration, mean ALT did not significantly decrease from baseline, but serum HCV RNA levels significantly decreased from 755.6 KIU/mL at baseline to 596.5 KIU/mL at the end of week 4, despite no patients having cleared HCV RNA. The mean Th1/Th2 ratio significantly increased from 15.9 at baseline to 17.6 at week 4 of RIB administration, suggesting that RIB administration has more effect on Th1 cell modulation than on Th2 cell modulation. During the combined IFN and RIB treatment, after 4 weeks of RIB administration, mean ALT and the Th1/Th2 ratio significantly decrease from the start of the combination treatment to the end of week 4 (from 80.7 IU/L to 49.4 IU/L and from 17.6 to 15.5, respectively). In 4 patients with virological response at 4 weeks after the start of the combined treatment, the Th1/Th2 ratio significantly decreased, from 16.3 at the start of the combined treatment to 8.4 at the end of week 4.
Conclusion: From these findings, the 4-week RIB administration resulted in a decrease in serum HCV RNA and increased Th1 cell modulation, which possibly led to the early clearance of HCV RNA seen when RIB alone was followed by combined IFN and RIB treatment.

P1680 Managing drug toxicities associated with pegylated interferon alfa-2b and ribavirin for the treatment of hepatitis C in patients coinfected with HIV

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Background: Morbidity and mortality secondary to HCV infection is of increasing concern in patients coinfected with HIV. Treatment with pegylated interferon (PEG-INF) and ribavirin (RBV) is currently most effective but is associated with significant adverse events.
Methods: We currently offer PEG-INF alfa-2b and RBV therapy to all coinfected patients in whom there are no contraindications. We describe a prospective review of adverse events and management of patients treated with PEG-INF and RBV in our unit.
Results: We have commenced treatment in 10 patients (seven hemophiliacs) and all have experienced adverse events. Six patients complained of symptoms of depression and five patients required antidepressant therapy after review by a psychiatrist. One patient temporarily discontinued therapy due to severe hemolytic anemia secondary to zidovudine in combination with RBV. Following a switch from zidovudine to tenofovir, PEG-INF and RBV were reintroduced without recurrence of severe hemolysis. Four hemophiliac patients have required G-CSF for treatment of neutropenia to enable HCV therapy to be continued at full dose. Dose modification of PEG-

INF alone did not maintain neutrophils >0.5 cells/mL. One patient developed diabetes requiring diet control. Other adverse events include weight loss, alopecia, thyroid function abnormalities, thrombocytopenia, ''flu like symptoms, splenomegaly, *H. influenzae* respiratory tract infection, dry skin and oral ulceration. No significant HIV related problems have arisen other than a mean fall in CD4 count of 119.45 (SD, 159.3) cells/mL, related to absolute reductions in white cell count. There have been no episodes of hyperlactatemia or symptomatic lactic acidosis. No patients had prolonged periods of absence from work and generally were able to continue with their activities of daily living as before.
Conclusions: Treating HCV in HIV coinfected patients is feasible but drug toxicities are very common. Patients with hemophilia are at increased risk of significant neutropenia. Patients must be well motivated and informed before embarking on treatment. Most adverse events can be managed safely without withdrawal of HCV therapy but a multi disciplinary approach with close monitoring is required.

P1681 The effect of combination interferon and ribavirin therapy in chronic hepatitis C

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Objectives: Combination therapy with interferon and ribavirin has been studied in many countries, i.e. virilogical respons has been reported approximately 50% in the USA. The aim of the present study was to evaluate the effect of combination therapy in Iranian patients.
Methods: Seventeen patients were studied. Three MU interferon Alpha-2b were injected subcutaneously 3 times a week in combination with ribavirin 1000–1200 mg daily per oral for 24 weeks. before The beginning of the treatment virus markers (HCV Ab, HCV RNA) and liver enzymes were recorded and liver biopsy were taken. These experiments were repeated after treatment too and the results were recorded.
Results: Among the 17 patients under study, 13 (57.1%), 14 (82.3%), and 7 (41.1%) had histological improvement, biochemical and virological response, respectively.
Conclusion: Six-month ribavirin, interferon therapy results in a significant reduction of virus load, histological improvement and biochemical response and thus it is a suitable treatment for patients with chronic hepatitis C.

P1682 Variability in HCV *E2* gene and treatment response

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Background: Because of the lack of a really effective treatment for hepatitis C virus (HCV) infection, the study of interferon resistance basis became crucial. In HCV *E2* gene two regions seem to be related with non response to therapy, the HVR1 and PePHD regions. The aim of this study has been to analyze the relationship between these regions' variability (expressed as quasispecies detection) and the viral behavior.
Patients and methods: Samples from 25 patients infected with HCV chronically were analyzed. The quasispecies were detected by nested RT-PCR following single-strand conformation polymorphism assay (SSCP). The bands were visualized by silver staining. Genotyping was performed using non type-specific primers of core region following hybridization with HCV subtype-specific 5′-biotinylated oligonucleotide probes to detect each subtype using DEIA (DNA Enzyme Immunoassay). The patients were divided into three groups with respect to response to therapy: eight patients with sustained response, nine patients with transient response and eight non-responders patients.
Results: The results have been different depending on the region. The variability in HVR1 varies according to the type of sample. In patients with sustained response a low pattern of heterogeneity was observed. In the rest of patients the genetic complexity pattern in HVR1 was high or the appearance of new bands was detected. Most patients involved in this study that showed an initial high genetic complexity pattern were infected with 1b genotype (85.71%) and those who showed a low genetic complexity pattern were 3a genotype (45.45%). By contrast, in PePHD region all samples presented a low genetic heterogenity pattern and it was independent of patients characteristics, as genotype, sex or age.
Conclusion: The effect of HVR1 region in interferon resistance could be related to the generation of new genetic variants able to escape from immune system, so the response is only achieved if the number of quasispecies in cell is low. But this possibility is not possible in PePHD region. This region does not present variability and is quite stable. This genetic homogeneity could be considered as an indirect signal of this region importance on viral persistence. Moreover, this study implies that the mechanisms used by these regions are completely different. However, more studies are needed to evaluate the real role of these regions from *E2* gene on the antiviral activated by the cell.

Tropical public health

P1683 Survey of Eltor cases in Iranshahr county 1996–2002

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Cholera is an acute intestinal infection caused by ingestion of food or water containing Vibrio cholera serogroup 01–0139. Vibrio cholera has been recognized as one of the most common causes of acute bacterial diarrheas throughout the world. During 1998, that was a dramatic increase in the number of cholera cases worldwide, as well as Asia, compared with 1997, with the total number of cases almost doubling. In Iran, the number of fatalities caused by the disease in the 1990s reduced to less than half of those reported in the 1980s and reached from 38,710 cases to 15,912. This reduction would be continued at the 20 decade. But the fatality cases reveal a periodically pick with alternation of 2 years. Iranshahr County is host of Afghan and Pakistani refugees. This county is struggling with drought for last 6 years. Thus, conditions would be prepared for some infectious disease as well as the Eltor. This retrospective study has conducted for survey of reported cases of the Eltor for identifying its risk factors in Iranshahr County. 3761 stool samples of the patients with diarrhea were studied during years 1996–2002. Majority of them were from villages around the county. 115 Vibrio cholera were isolated with an isolation rate of 3.05%. Fatality rate was 4.35%. Carry Blaire and TCBS cultures were used as the gold standard for diagnosis of cholera. 104 cases (90.5%) of these were Ogawa serotype and rest 11 (9.5%) was NAG vibrios. All the patients (100%) presented with loose stools. 11.3% of affected individuals were children below 2 years, 32.17% between 2 and 5 years, 22.6% between 5 and 10 years, 33.91% up to 10 years of age. There were 67.5% males and 32.5% females. The patients were using various systems of water supply (31.3% from ducts, 36.5% from tap water and rest 32.2% from the other sources, e.g. wells, springs, rivers and so on). It is obvious that the water supply and its hygiene in villages of this county needs a basically revision. Training of villagers also would be very useful.

P1684 The health-seeking behavior of parent/carer of severe malaria children before admission

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From January 1st to August 31st 2000, we carried out a cross-sectional study of parents/caretakers of 148 children aged 6–59 months, hospitalized for severe malaria, at the Djoungolo and the Mfou district hospitals, in order to describe their health seeking behavior. The mean delay between the onset of illness and arrival at hospital was 3.9 days. Most (79.7%) parents/caretakers resorted to auto-medication. Antimalarial drugs were most presumedly used (76.6%), amongst which chloroquine was the most frequent (73.4%). Only 51.1% of the antimalarial drug were presumedly administered at adequate dose. We

recommend that for better management of malaria at home, health education of parents/caretakers should be intensified.

P1685 Study on the relationship of zinc and iron serum concentration with giardiasis in children under 12 years of age, referred to hospitals affiliated to the Mazandaran University of Medical Sciences

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Objective: Giardiasis is one of the most prevalent human intestinal parasites, particularly in children. Its presence in the intestine causes damage to the deudenum and jejuneum inner cellular wall, which results in malabsorption of different important substances such as zinc and iron which can lead to the delay in physical and mental growth, anemia, diarrhea. Since the absorption of these two elements are trace and are dependent on the certain existing conditions, these trace elements are essential, and if their absorption is inhibited, the complications will appear soon. The aim of this research is to determine the serum concentration of zinc and iron in the children infected with *Giardia lamblia*.
Methods: 100 children under 12 years of age were chosen. The presence of *Giardia lamblia* in stool samples was identified by direct and formal ether methods. This group of patients had no other infectious diseases. The control group consisted of 100 children under 12 years of age without any clinical and laboratory findings. 5 mL of blood was drawn from each person and serum was separated. Questionnaire regarding the age, physical growth status and clinical condition was prepared and filled. Serum zinc and iron concentration was measured with atomic absorption spectrophotometry. A questionnaire related to the age, height, weight, parents' level of education, the kind of residential areas, the drinking water, the clinical characteristics of Giardiasis was filled.
Results: In this study, the mean and standard deviation of serum iron in test and control groups were 0.67 ± 0.25 ppm and 0.77 ± 0.23 ppm, respectively, and for serum zinc concentration in the test and control groups were 0.64 ± 0.20 ppm and 0.96 ± 0.23 ppm, respectively. On the basis of the *T*-test, there was a significant differences between the test and control groups on their serum Iron concentration ($P < 0.01$). Also there was statistically highly significant differences in zinc serum concentration ($P < 0.001$) between the case and control groups.
Conclusion: Considering the above mentioned results, in order to prevent the deficiency of above mentioned elements and prevention of the arisen complications, the consultant physicians are recommended to change the therapeutic strategies and control the blood factor and also the pharmaceutic factories are recommended to add zinc and iron as complement to the antiparasitic drugs, to help to improve the society welfare.

P1686 Environmental pollution with soil-transmitted parasites in a healthy village (Kargan) before environmental sanitation, Ardabil, Iran

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Objectives: Some of parasitic diseases especially intestinal parasites transmitted by water, soil and food are the most important economic and healthy difficulties in the developing countries. Aim of this study was to determine the sources and rates of parasitic infections in Kargan village, Ardabil.
Methods: In this cross-sectional survey 331 stool samples from village inhabitants, as well as 30 water samples, 30 waste water and 40 soil samples from the village were collected and examined for the presence of parasitic eggs and cysts.
Results: 49.8% of the stool samples were infected by at least one type of intestinal parasite. 16.6% of the water samples, 42.5% of the soil samples and 36.6% of the waste water samples were found to be positive for parasitic eggs and cysts.
Conclusions: These results indicate that soil and waste water are heavily contaminated and parasites are circulated between human and the environment. Improving environmental sanitation is imperative for the control of soil-transmitted parasites in this village.

P1687 The outbreak of toxoplasmosis in pension students in Turkey

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Objective: Human toxoplasmosis originating from infected cats with *Toxoplasma gondii* is a major public health problem. It has been known that majority of human infections with *T. gondii* have a benign course in immunocompetent subjects. There are several well-documented reports about some outbreaks of acute toxoplasmosis. Over the last 10 years the serologic diagnosis of infection has been based mainly on the enzyme-linked immunosorbent assay (ELISA). Also, ELISA IgG avidity and VIDAS tests are in use widely. The aim of this present study was to investigate and to evaluate and to report the largest outbreak of toxoplasmosis by different serologic tests (ELISA IgG/IgM, IgG avidity, and VIDAS IgM) in the reported studies so far.
Methods: A total of 56 male students who live in a pension high school aged between 14 and 18 years. They presented with mostly flu-like symptoms (subfebrile/fever, myalgia, dizziness, headache) with/without cervical lymphadenopathy. For the investigation, serologic test such as ELISA IgG/IgM (Equipar, Italy) IgG avidity (Bouty Beia, Italy) and bioMérieux Vitek VIDAS Toxo IgM (France) were used.
Results: Based on serologic tests, all of the reported patients were seropositive for *T. gondii* antibodies by ELISA IgG/IgM, and VIDAS IgM. Additionally, 36 (92.3%) of 39 had low IgG avidity and only 3 (7.7%) of 39 displayed discrepant IgG avidity. All of patients were found with LAP (cervical/submandibular/retroauricular/suboccipital, ca. 1 cm × 1.5 cm size) and otherwise healthy when examined by infectious disease physicians. Also, none of the student had evidence of an active chorioretinitis focus in retinal examination by ophthalmologist.
Conclusion: Acute toxoplasmosis outbreak was diagnosed according to the results of presence of anti-*T. gondii* antibodies by serologic tests (ELISA IgG/IgM, IgG avidity, and VIDAS IgM). The data suggest that the ELISA IgG avidity test can be helpful and useful tool in differentiation of recent and past toxoplasmosis in epidemic settings. No treatment was given to any student because of healthy immune system and lack of symptom. It remains unclear (or mystery) how could this toxoplasmosis outbreak occur in spite of much efforts in searching possible transmission ways (drinking water, cats, etc.). But this population will be resolutely continuing the follow up research periodically both serologically and physically.

P1688 Malaria chemoprophylaxis for international travelers in Bahrain

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Objective: Inappropriate use of malaria chemoprophylactic regimens is considered one of the contributing risk factors for acquiring malaria by travelers destined for endemic areas. We assessed the malaria chemoprophylaxis practice in Kingdom of Bahrain.
Methods: A descriptive, questionnaire-based prospective study conducted between July 2001 and June 2002 in 43 out of a total 50 private-sector pharmacies.
Results: A total of 201 responses were received by the end of the survey from 23 pharmacies. Among 201 individuals 144 (71.6%), 52 (25.9%) and 5 (2.5%) sought antimalarials for chemoprophylaxis, treatment, and for standby treatment purposes, respectively. Antimalarials were most commonly dispensed during June and July. Chloroquine was the most commonly dispensed antimalarial prophylactic (61.1%), followed by mefloquine (27.8%). In 144 individuals who sought chemoprophylaxis and travelled to endemic areas, 101 (70.1%) were provided with inappropriate chemoprophylaxis. Of these, in 73 antimalarials were dispensed for self-medication purpose, whereas, in 20 in response to advice/prescriptions issued by public health advisory body, and in 8 in response to private clinic-based prescriptions.
Conclusions: We found a considerable disparity between malaria chemoprophylactic drugs dispensed and regimens recommended by the World Health Organization (WHO); more than two-third of travelers who sought chemoprophylaxis received inappropriate drugs. In order to optimize malaria chemoprophylaxis and to encourage travelers to seek proper medical advice, antimalarials should be no longer dispensed on an over-the-counter basis, and chemprophylaxis advice should be provided only by the public health advisory body and by physicians.

Public health: knowledge, attitude and impact

P1689 Development of a questionnaire for assessing medical awareness on bioterrorism issues

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In the aftermath of the anthrax outbreak of October 2001, we attempted to assess the awareness of medical graduates and young doctors in our area over basic issues of bioterrorism. We used a multiple-choice questionnaire focusing on basic, historical, taxonomic, epidemiologic, clinical, and treatment-related issues of the usual biological warfare. The results concluded that awareness of young doctors over matters of bioterrorism is only marginal. The median score of correct answers was only 38%, falling in the predetermined 'poor' category. The questionnaire was also used as a tutorial on basic aspects of biological weapons: its results moreover emphasized the need for enhanced medical alert over such issues, in order to minimize the unavoidable media-fuelled frenzy that accompanies such dire events.

P1690 Sexually transmitted infections and prenatal control: health services characteristics and user satisfaction

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Aims: In this work one evaluates the satisfaction obtained by the users of the prenatal control services (PC) and sexual transmitted infections (STI) as well sexually comparing itself with some characteristics of the supplier and the basic characteristics of the service of health.
Material and methods: A cross-sectional survey was applied in where 248 pairs of supplier–users of health services in 95 units of attention of first level of eight states of Mexico were obtained. The information was obtained by means of the direct observation of the medical consultation, interviews to directors, suppliers, users and of the application of an examination of knowledge to the suppliers. The collected data were analyzed using logistic regression in where the association between the pertinent formation of the suppliers was analyzed, the treatment that they gave him to the patient in the consultation and the satisfaction that the users declared.
Results: The satisfaction of the users of the services of PC and STI is associate to the treatment received in the consultation and to the time that hoped to receive it, but not to the preparation adapted of the supplier nor to its age or gender. The treatment received by the user also was associated with its socioeconomic level, being that the poor users but receive a worse treatment proportionally.
Conclusions: The evaluation of the satisfaction of the patients can be fundamental to improve the decision making in the design, organization and operation of health services, in special in those places in which it exists shortage of resources and/or conditions of economic inequality. In these cases the benefit of the health care services can deepen the differences between the population, affecting more to the poorest population.

P1691 Study of knowledge, attitude and practice of health personnel of Iranshahr district about the education methods of malaria prevention

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Objectives: To describe Iranshahr health personnel's knowledge, attitude and practice about education methods of malaria prevention and determine which factors influence the use of methods for malaria prevention education to the people.
Methods: In January–December 2002, a cross-sectional survey of 144 health personnel of rural health centers of Iranshahr District (Sistan and Baluchestan province, souteast Iran)was conducted with respect to malaria education methods.
Results: The KAP of 66.1% of health personnel was relatively average. (Mean $\pm$ SD),the KAP of 18.8% was low and 15.1% was high. There was no significant relationship between the KAP and the variables: age, sex, literacy, marriage, ability to speak in local language, record of service, the number of under protection population, presence of educational activity reporting system and kind of multimedia educational means ($P < 0.05$).
Conclusion: The health personnel were more able to educate by using face to face (using picture) method. Such methods are more effective in increasing knowledge. For effective change of the attitude and practice, the methods such as asking and answering, group discussion, ... are more effective. Due to the results, they believe that the present methods are repetitious, not interesting and nonsuitable methods and multimedia education means in the rural health centers are not enough.

P1692 Medicine management and public health

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The antibiotic resistance represents an important problem of public health and we have to find all the ways to control it. In a previous work, presented at the ECCMID Congress in Milan, we have found the self-medication tendency of the public from our city and now we continue the study, searching the self medication causes. In order to determine this we elaborate a specific questionnaire formed of 6 questions and we applied it to three homogenous groups: patients, physicians and, pharmacists, 70 of each category, from our town. The work contains a great number of figures (graphs) presenting the frequencies obtained for the answers. The results evidential that all the respondents appreciate the following causes: difficult access to health services, big taxes for health insurance, big expenses for medical assistance and also small prices of medicines, as being important for the self medication tendency of the public. A very important question was about other causes of the self medication, when the patients answered the following: advice from other people, influence of nonprofessionists, lack of health education, big prices of medicines, lack of time, fear of medical procedures. We were interested to find what is the pharmacist role in self medication control and we applied a specific question, obtaining special results. At the end of the paper we analyzed the National Formulary 2002 about the dispense of medicines without prescription. The law is very firm: 72% from the pharmaceutical products are delivered only with medical prescription and 28% without it. In conclusion, the pharmacist can control the self medication tendency of the public and this is very important for the case of the antibiotics, being a way to decrease resistance.

P1693 Provision of communicable diseases screening and vaccination services to asylum seekers in Ireland

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The number of AS applicants to Ireland has dramatically increased in recent years with over 10 000 applicants yearly since 1999. There is a national policy of Dispersal and Direct Provision of asylum seekers (AS) to centers throughout the country where accommodation, food and a small weekly allowance are provided. Communicable Diseases (CD) screening and vaccination service is offered to all AS on a voluntary basis within the centers by a mobile team of public health doctors and nurses with clerical support. A hospital-based liaison nurse facilitated referrals from screening program to specialty services as required. By January 2003, 4323 AS were dispersed to 18 accommodation centers in the Southern Health Authority region in Ireland. We prospectively collected data on all AS attending the CD and vaccination service over a 6-month period July–December 2002 and entered the data into a modified occupational health database. Over 90% AS are from the former Soviet Republics and sub-Saharan Africa and 45% are female. Interpretative services were required for 3.5% of consultations. 3047 appointments were sent with 2443 (80%) attendance's of which 577 were new to the service. Of the 577 new attendees, 575 (99%) completed screening for TB with no evidence of active infection, 520 (90%) accepted testing for Hepatitis B virus and 517 (89%) for HIV. Pregnancy rate was high at (7%). Only 6% had a record of vaccination and 86% was deemed to require further vaccination, which was completed by the mobile team. Mobile units of health professionals providing services within accommodation centers improved the uptake of CD screening and vaccination. Hospital-based liaison nurse working with these units facilitated rapid referral of AS from screening services to specialty services as required. Vaccine uptake and completion was high.

P1694 Impact of conjugated meningococcal C Vaccination in the Netherlands

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Objective: In response to increasing incidence of meningococcal C disease since 2000, regular conjugated meningococcal C vaccination was implemented in September 2002 for children aged 14 months. A catch up campaign was carried out (coverage ± 90%), targeting children aged 1–5 and 15–18 years in June–July 2002 and those aged 6–14 years in September–October 2002. To monitor the impact of vaccination (intensified) meningococcal disease surveillance was performed.
Methods: Isolates from patients with meningococcal disease collected by the Netherlands Reference Laboratory for Bacterial Meningitis were analyzed from 1993 onwards. At the end of 2002, intensified meningococcal disease surveillance was started using an electronic questionnaire linked to the compulsory notification system.
Results: The incidence of meningococcal C disease increased from 0.5/100 000 in 1993–1999, to 0.7 in 2000 and 1.7 in 2001, representing 13%, 19% and 38% of all meningococcal isolates (remainder mostly serogroup B). Highest incidences in 2001 were reported for those younger than 19 years, with a peak at 15–18 years (8.4/100 000) and 1–5 years (7.2). The incidence of meningococcal C disease amounted to 1.3/100 000 in January–November 2002. The highest incidence was reported in the months January–March 2002 (3.1, 2.8, and 2.2/100 000, respectively) and decreased afterwards to 0.1 in November. Simultaneously, a seasonal decrease of serogroup B disease occurred. Since the start of vaccination only a few (unvaccinated) cases were reported among the age groups targeted for vaccination.
Conclusions: The increasing incidence of meningococcal C disease in 2000 and 2001 prompted a nationwide vaccination campaign from June 2002 onwards. It is not yet clear to what extent the observed decrease in incidence during 2002 is due to vaccination or seasonal variation. The impact of meningococcal C vaccination will only be evident after the annual seasonal peak in meningococcal disease. Surveillance data of the first months of 2003 will be used for this purpose.

P1695 Evaluation of the National Meningococcal C Vaccination Campaign, client satisfaction and logistics

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Objectives: A national vaccination campaign against group C meningococcal (MenC) disease was held from June–December 2002. 3.6 million children aged 1–19 year were eligible for vaccination with a menC-conjugate vaccine. This campaign was the largest ever held in our country and resulted in >90% coverage. The national association of Municipal Health Services (MHS) was responsible for the organization and coordination of the campaign while the local MHS's carried it out, in cooperation with national institutes (NI) like the NI for Public Health and the Environment (RIVM) and the National Co-ordination Centre for Communicable Diseases (LCI). An evaluation study was set up addressing the logistics and subject satisfaction.
Methods: Two questionnaires were developed, 1 to evaluate the logistics and 1 for subject satisfaction. All 40 MHS's in the existing seven regions received the two lists. The MHS's distributed >17 000 subject lists among the vaccinated subjects. The data of the MHS's were put together per region for analyzes.
Results: Thirty-four logistic and 8141 subject lists returned, response was resp. 85 and 48%. Subject satisfaction results showed that of the vaccinated subjects, 5–15% doubted the benefit of the intervention. Doubters sought significantly more information about the vaccination at forehand than nondoubters. To search extra information, 0–4% hit the specific websites about the campaign. Between 41 and 59% experienced post vaccine complaints, of which pain at the site of vaccination or pain in the limbs were most frequent. Subjects indicated to be very positive about the campaign (89–100%). Logistic results indicated that MHS's organized the campaign according to the central guidelines but peripheral adjustment were made, dependent on the local situation. Some decided to vaccinate all children at one day on one site, others arranged over 20 session at different days. In all cases subjects and personnel were positive about the organization. Multiple vaccination sessions resulted in high operation costs. MHS's experienced the central support of the national association of MHS as crucial.
Conclusions:
1. Subjects experienced the organization of the intervention as positive.
2. Uniform evaluation resulted in best practice recommendations for future mass campaigns, however, local MHS's must respond to local circumstances

P1696 Community involvement with young orphans infected and affected by HIV/AIDS. Prevention and care programs as a way forward to fight stigma and discrimination

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Objectives: The community and relatives of AIDS orphans disassociated themselves in caring for AIDS orphans because of stigma and discrimination.
Methods: Five years ago, the burden of caring for AIDS orphans was left to AIDS Service organizations. The community that includes political and religious leaders, family members discriminated AIDS orphans due to stigma associated with HIV/AIDS. This was due to lack of information about HIV/AIDS or lack of knowledge about the disease and what is involved in caring for War orphans and AIDS orphans. The community and relatives disassociated themselves from the responsibility of caring for AIDS orphans. Two years ago, we embarked on building the capacity of community in the prevention of HIV and care for orphans infected and affected with AIDS in communities and were mobilized, sensitized and trained in their role in prevention and care right from the subcounty to the village level. Orphanage support communities were established in two subcounties of Mateete and Luwebitukuli in Ssembabule district.
Results: Orphanage support committees were formed, i.e. subcounty orphanage support committees and parish committees, 156 clients accepted to help these children to outreach centers, which were opened in the community. Community members offered their own home to be used as out-reach centers. The numbers of orphans who are not HIV positive are accessing medical services at our Center, it was unheard of in the past. The number of orphans reporting for VCT services is increasing, i.e. November 2001–June 2002, 1205 orphan clients reported for VCT at the centers and in the community out-reaches, orphans and other people were willing to offer their homes as out-reaches for treatment and VCT, couples accessing VCT together has also increased unlike in the past. HIV positive couples actively involved in HIV/AIDS drama, orphans' drama group has emerged to further reduce stigma and discrimination in the community.
Conclusion: Involvement of the community in HIV/AIDS prevention and care reduce stigma and discrimination, hence promoting behavior change, positive living, pro-longing lives for orphans reducing stigma and discrimination enhances quality of life for orphans and PHAs do not mind the project vehicles labeled with the word AIDS Services. The government should encourage the information of orphanage support committees from subcounty to the village level. This will encourage people to break the barriers of effectiveness in HIV/AIDS prevention and care. Only by confronting stigma and discrimination will the fight against HIV be won.

P1697 Prevention of rabies in Georgia

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Background: Animal bites are a common but under-recognized public health problem. It has been estimated that there are 8–10 000 bites each year in Georgia, and based on an average visit and postexposure treatments cost at list $120 000 per year. Despite the frequency and expense of these injuries, there is little information about the incidence of animal bites because of a lack of systematic reporting and a lack of measurement of the quality and completeness of reported data.
Objectives: To investigate animal bites and rabies reported cases, reveal unreported cases, analyze and based on study results find more effective epidemiologic measures of animal bites and deaths (due to rabies) prevention in Georgia.
Methods: The capture–recapture method was used, along with log-linear modeling. Four sources were used to identify victims: policlinic/ambulatory reports, hospital reports, animal control reports and victim reports.
Results: In 1980–2001, 150 700 dog and other animal bites were reported. The capture–recapture method estimated that there were 146 200 unreported bites. During these period, 118 deaths due to rabies were registered in Georgia and 67 (57%) cases among them have been registered during the last 6 years.

The reasons of fatal cases were untreated (47%), uncompleted treated (34%) and late began postexposure treated (19%) cases of bites (mostly dog bites). About 56% of bitten people did not know about rabies and its prevention measures. About 32% had incorrect information about prevention and only 12% of them knew epidemiologic and clinical aspects of disease. About 16% of physicians who were responsible for quality postexposure treatment had not an adequate knowledge.
Conclusion: Dog and other animal bites are common but preventable injuries. To improve surveillance and prevention of rabies in Georgia, the focus should be on educating the general public about the serious consequences of animal bite injuries and developing the animal's vaccination strategy.

P1698 A south-east London outbreak of *E. coli* 0157:H7 in a day nursery

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Objectives: This poster reports an outbreak that took place in a South-East London Day nursery between the latter part of June/early July to mid August 2002. Microbiological evidence confirmed 15 cases from amongst the nursery children and their contacts. Environmental Health reported a further four probable cases and six possible cases based on their investigations.
Methods: The outbreak was recognized on 2 July 2002, when *E. coli* 0157 was isolated from two samples coming from separate GP surgeries in the same area. Initial inquiries indicated that both children attended the same day nursery. All the nursery children and staff were screened revealing that 10 children were carriers of which five were asymptomatic. Three household contacts were also found. All 15 strains isolated, were serotype positive for *E. coli* 0157: H7 using the *E. coli* 0157 Latex test (Oxoid) in the laboratory. All 15 strains phage typed 21/28. All strains were positive for Verotoxin genes. Only one of the 15 nursery children was hospitalized overnight for i.v. dehydration. None of these children or their contacts was treated with antibiotics. None of them developed hemolytic uremic syndrome or any other serious complications. Environmental Health investigations identified inadequate toilet facilities but were unable to trace the source of the infection.
Conclusions: The day nursery was closed and the toilets refurbished. The children and staff were allowed back after two negative stool cultures. However further testing indicated some intermittent shedding after two negative stool cultures and highlights the fact the protocol may need reviewing.

Protozoan infection – clinical aspects

P1699 Kala-azar in Sheffield, UK

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Introduction: Visceral leishmaniasis is a major cause of morbidity in tropical countries and, untreated, has a high mortality. Leishmania is endemic in Southern Europe. It can present atypically depending on the host cellular immune response. Pancytopaenia and hepatosplenomegaly are recognized features. Serology is used to aid diagnosis, but both false positives and false negatives can occur and be misleading. We review 3 challenging clinical cases with interesting serological results. Case 1: A 51-year-old man who had lived in Malta gave a 1-year history of a peri-anal lesion. Biopsy was suggestive of Crohn's disease; treatment was with oral prednisolone and azathioprine. 18 months later he presented with persistence of the peri-anal lesion, hepatosplenomegaly and pancytopaenia, thought to be azathioprine induced. However, a bone marrow examination revealed Leishman–Donovan bodies and symptoms resolved on amphotericin. Case 2: A 60-year-old male Maltese resident presented with fever, hepatosplenomegaly and pancytopaenia. Initial investigations including serology and bone marrow examination were negative for leishmania. The blood picture was attributed to splenomegaly secondary to alcoholic liver disease. Repeat investigations in Sheffield showed amastigotes in the bone marrow and serology was positive. Symptoms and hematologic indices improved on treatment. Case 3: A 61-year-old lady who had lived in South Africa presented with malaise, splenomegaly and pancytopaenia. Initial leishmania serology was strongly positive, but lymphocyte markers and bone marrow biopsy were suggestive of lymphoma. Repeat bone marrow was again consistent with lymphoma; no amastigotes were seen. The patient had a raised IgM; further serological assays on IgM depleted serum were negative for leishmania antibodies.
Discussion: These cases highlight the importance of considering leishmaniasis even if patients have never left Europe. Reliance on serological tests to make or refute a diagnosis can lead to errors. Increased awareness of the geographical distribution and atypical manifestations of disease are key factors in establishing a diagnosis.

P1700 Spectrum of autoimmunity and dysproteinemia in patients with visceral leishmaniasis

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Aim: To describe five patients with visceral leishmaniasis (VL), who presented with fever, dysproteinemia and a variety of autoimmune manifestations that were initially misdiagnosed as having an autoimmune disease.
Case reports: The first patient was a 43-year-old woman who presented with fever of four weeks' duration, arthralgias, splenomegaly, and palpable purpura on the legs. Laboratory investigation revealed marked polyclonal hypergammaglobulinemia (gamma globulins 7.2 g/dL), decreased C4 fraction of the complement (<8 mg/dL), high titers of serum rheumatoid factor (RF), antinuclear antibodies (ANA) and cryoglobulins, as well as positive direct Coombs' test. The second patient was a 72-year-old woman, who presented with prolonged fever, marked splenomegaly, severe anemia and thrombocytopenia, positive direct and indirect Coombs tests, positive ANA, elevated levels of γ2-microglobulin (21026 μg/L) and biclonal hypergammaglobulinemia. The third patient was a 24-year-old man, who presented with fever, splenomegaly, pancytopenia, positive ANA, and monoclonal (IgG-β) hyperglobulinemia. The fourth patient was an 80-year-old woman with fever of one month's duration, splenomegaly, decreased C3 and C4, high titers of RF, ANA and cryoglobulins, as well as positive antithyroid, antismooth muscle cell and antineutrophil cytoplasmic autoantibodies. The last patient was an 29-year-old woman, who presented with fever, positive RF, and positive ANA. In all cases VL was diagnosed by the presence of intracellular parasites in bone marrow aspiration and high titers of antileishmania antibodies in serum. Six to 12 months after the initiation of appropriate therapy a nearly complete recovery of the immune abnormalities was recorded.
Conclusion: Autoimmune manifestations are not uncommon in patients with VL and can lead to the misdiagnosis of an autoimmune disease. The initiation of immunosuppressive therapy may have deleterious consequences in such patients. VL should be included in the differential diagnosis of febrile patients with evidence of autoimmunity.

P1701 Traveler returns with visceral leishmaniasis

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An interesting case of a 32-year-old gentleman who presented with a PUO and subsequently developed the classical pentad of visceral leishmaniasis: fever, weight loss, splenomegaly, pancytopenia and hypergammaglobulinemia. He took a year out to travel to South and South-east Asia, Australia and New Zealand and Central America. He traveled on a budget staying in basic accommodation, eating out at street stalls and local restaurants. He avoided high risk activities but did have an unprotected liaison in Laos. He was in contact with local cats, dogs and bird life but the only bites he sustained were that of insects. He did trek in the bush often in open-toed sandals and slept under the stars. He returned to the UK with a 4-week history of fever, drenching sweats, dry cough and anorexia. On initial presentation he was found to be spiking fevers up to 39°C, anemic, leukopenic and with splenomegaly of 15 cm. His CRP and ESR were elevated and he had deranged liver function with elevated transaminases. The initial differential diagnoses were that of malaria, typhoid, respiratory infection or viral hepatitis. His fever settled with paracetamol but his liver function remained abnormal. Malaria films, cultures and serology were all negative. With follow-up he developed progressive pancytopenia with his hemoglobin falling to 7.1,

neutropenia 0.4, platelets 40 and with elevated CRP, ESR and ferritin. He had further enlargement of his spleen and lost nearly 20 kg in weight over a 6-month period. Additional investigations had included negative Leishmania DAT and latex, negative bone marrow on two occasions, evidence of hypergammaglobulinemia with DAT positive anemia. Serology and cultures were negative for tuberculosis, histoplasmosis, brucella, cryptococcus, rickettsial disease, flaviviruses, HIV, HTLV1, coxiella, coccidioides, yersinia, CMV, parvovirus, and positive serology for EBV, Sin Nombre hantavirus and toxoplasma, although it was felt that these results did not explain his clinical picture. The diagnosis was subsequently confirmed on Leishmania serology (DAT and rK39 antigen test) and repeat bone marrow aspirate and trephine. It was thought that he had acquired his leishmaniasis whilst visiting Chitwan National Park in Nepal some 5 months before presentation. The species was Leishmania donovani and he responded quickly to liposomal amphotericin.

	Progressive pancytopenia					
	20 Mar	26 Mar	29 Apr	2 May	20 May	18 Jun
WCC	1.8	2	1.3	1.2	1	1.7
Neutro	0.9	0.8	0.6	0.6	0.5	0.3
Hb	7.6	7.1	6.8	5.9	8.1	7.2
mcv	69	70	75	75	75	71
Plt	176	157	153	139	86	105
ESR		74	110		55	85
Femitin		2313				5390
CRP	71		124	105	114	99
ALT	58	61	27	28	62	66
GGT	66	55	23	28	65	38
ALP	133	120	65	74	190	165
Bil	13	20	9	9	6	12
LDH			956			
Alb	27	27	27	27	20	18

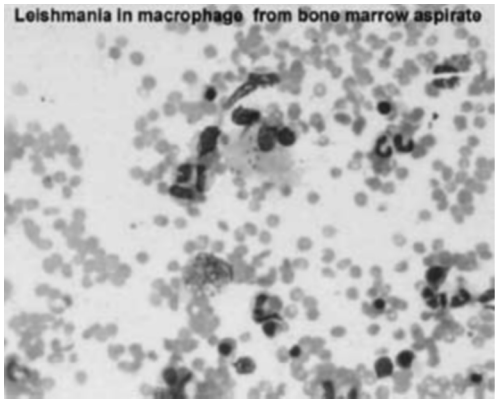

Figure 1

P1702 *Entamoeba histolytica* presenting like acute appendicitis

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A 24-year-old man was admitted to our hospital due to a cramp like pain in the right lower abdomen, which was accompanied by vomiting and diarrhea. The diarrhea started 4 weeks ago while over the next few days his symptoms persisted and his general state of health deteriorated. He suffered from mild to moderate dehydration and he experienced recurrent episodes of diarrhea, intense abdominal cramps with rebound tenderness and vomiting stimulated by eating. Hematological investigation measurements of erythrocyte sedimentation rate tests of liver and renal function were within normal ranges. The major concern about this patient was the possible inflammation of the appendix since his clinical signs mimic acute appendicitis along with/or inflammatory bowel disease. Fibreoptic colonoscopy examination was done and multiple biopsy samples were taken that revealed normal colon mucosa. CT abdominal scan was not determined lymphadenopathy or other solid organs abnormalities. Extensive and more detailed history revealed that the patient had traveled in tropical countries 6 months ago. Parasitologic examination of the feces was unable to detect cysts and/or trophozoites of parasites while the serologic examination revealed antibody title of *Entamoeba histolytica* IgG: 1/160 and IgM: 1/640. The patient was placed on metronidazol 750 mg three times per day for 10 days and fluid replacing treatment. A great improvement of symptoms was observed within the following few days and he was discharged from the hospital. It is concluded that patients with symptoms of acute appendicitis after travel in tropical areas should be screened for *Entamoeba histolytica*. Although the demonstration of hematophagous trophozoites in feces is the definite method of diagnosis unfortunately the test has many drawbacks. Serology can support the diagnosis and a positive test is highly predictive of current disease.

P1703 Transfusion-transmitted malaria

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Malaria is a rare but potentially serious complication of blood transfusion. One case of transfusion-transmitted malaria was presented here. A 47-year-old woman came to our clinic with fever, diarrhoea, vomiting, and she was hospitalized. She had total hysterectomy and bilateral salpyngoooforectomy operation, and taken 1 U blood transfusion before 10 days of admission. One day before admission, chills, fever, diarrhoea and vomiting began. Patient's body temperature raised 39.5°C every night and lasted 6-h period during hospitalization. Laboratory results of the patient are: anemia, elevated lactic dehydrogenate and hyperbilirubinemie, other parameters were normal. The diagnosis was made examining blood smears with shown of *Plasmodium vivax* trophosoids. Chloroquine and primaquine was initiated for therapy and the patient was successfully treated. We learned that the donor made his military service in an endemic area for malaria, but we could not reach him. Because our region (North-eastern of Turkey) is not an endemic area for malaria and the patient had no travel history to an endemic area, we considered transmission route of malaria is blood transfusion in this case. The case illustrates the importance of considering malaria in diagnosing a febrile illness following blood transfusion in any patient. Because no approved tests are available to screen donated blood for malaria, prevention of transfusion-transmitted malaria requires careful questioning of prospective donors.

P1704 Comparison of efficacy of single dose of tinidazole with metronidazole (standard dose) in giardiasis: preliminary report

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Hamadan, IR

Objective: *Giardia intestinalis* is the most common intestinal protozoa in the under developing countries. Treatment of infection has some difficulties by metronidazole because of long course of therapy and some side-effects. The object of this study was to determine efficacy and side-effects of tinidazole in treatment of *G. intestinalis* infection. This is preliminary report of an ongoing trial.
Methods: A randomized controlled trial, 47 subjects with *G. intestinalis* infection were treated with tinidazole or metronidazole. Tinidazole 50 mg/kg single dose and metronidazole 25 mg/kg three times a day for 7 days were given orally to 24 and 23 children, respectively. Parasitological cure was documented when there was 3 times negative stool examination for *G. intestinalis* at 1–2 weeks after therapy.

Results: Twenty-one of 23 individuals treated with tinidazole and 20 of 24 children treated with metronidazole had parasitological cure. Cure rates between two groups was not significant statistically. No major side-effect were observed except one case in metronidazole group who had mild headache and abdominal pain for 2 days.
Conclusion: We concluded, tinidazole, at the protozacidal lable dosage, has efficacy equal of metronidazole in the treatment of *G. intestinalis*. Because of single dose administration, short course of therapy and good compliance of patients, this preparation is preferred to metronidazole in *Giardia* infection treatment.

P1705 Comparison of short course therapy of giardisis with standard dose of metronidazole: a randomized clinical trial

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Background and objective: *Giardia intestinalis* is the most common intestinal protozoa in the under developing countries. Treatment of the infection by metronidazole; this first choice drug, has some difficulties because of long course of therapy and some side-effects. The objective of this study was to determine efficacy and side-effects of short course treatment of the *G. intestinalis* infection by metronidazole vs. the long course (standard dose) of this drug.
Patients and methods: In a randomized controlled clinical trial, 54 subjects with *G. intestinalis* infection were treated with metronidazole. Metronidazole 15 mg/kg single dose for three days were given for study group and, 5 mg/kg three times a day for 7 days for control group were given orally to each 27 children, respectively. Parasitological cure was documented when there was 3 times negative stool examination for *G. intestinalis* at days 7, 10 and 14 after therapy.
Results: Eighteen out of 27 individuals (66.6%) cured with metronidazole in the both groups (short course and standard dose) and had parasitological cure as well. Cure rates between two groups was not significant statistically. No major side-effect were observed in the long course group but, abdominal pain (five patients), vomiting (three patients) and diarrhea in asymptomatic cases (two patients) observed in the short course group.
Conclusion: This study indicated that, the metronidazole in a short course regimen has efficacy equal of metronidazole, standard dose, in the treatment of giardiasis. Because short course administration is easy and has good compliance of patients (except some mild side-effects) this regimen could recommend in treating giardiasis.

European study group corner: Presentation of ESCMID Study Groups and Related Organizations

P1706 ESCMID Study Group on Antibiotic Policies (ESGAP)

H. Richet, B. Cookson, D. L. Monnet, I. C. Gyssens, F. M. MacKenzie, M. Cizman, M. Lelekis, H. Westh, I. M. Gould on behalf of ESGAP

ESGAP was founded in March 1999 to provide a uniting European forum for those actively involved in antibiotic stewardship at local, national and international levels. Its practical objectives are: (i) to promote awareness of antibiotic misuse and a better understanding of the factors involved (ii) to provide an opportunity for training in the appropriate use of antibiotics (iii) to facilitate collection and establish comparability of antibiotic prescribing data within Europe (iv) to identify problems of antimicrobial resistance related to antimicrobial use and to formulate strategies of antibiotic stewardship designed to control resistance, and (v) to promote the development, implementation and standardization of antibiotic policies at country level and in single institutions. ESGAP activities are presented on its Internet site and promoted through various courses, workshops and symposia arranged by ESGAP, in collaboration with other ESCMID Study Groups or other organizations. The proceedings of a symposium at the 11th ECCMID, which include guidelines on how to develop an antibiotic policy, were published in Clinical Microbiology & Infection (2001; 7 Supplement 6). In 2003, ESGAP will arrange the 22nd ESCMID Post-Graduate Education Course on 'Measuring, Auditing and Improving Antimicrobial Prescribing' and a workshop at the 43rd ICAAC on 'Monitoring and Evaluating Antimicrobial Use in Health Care Facilities'. Finally, ESGAP runs, together with three other ESCMID Study Groups, the 'Antibiotic Resistance Prevention and Control' (ARPAC) project funded by DG-Research of the European Commission. Within the project, ESGAP is responsible for the collection and analysis of data on antimicrobial use and antibiotic policies in participating hospitals. To facilitate standardized data collection, ESGAP developed an Antibiotic Consumption Calculator (ABC Calc) to transform aggregated data provided by hospital pharmacies into meaningful antimicrobial utilization rates. The 2003 version of this simple computer tool is freely available on the ESGAP Internet home page. New activities in 2003 include the establishment of an international exchange grant (ESCMID News 2002; no. 3) and teaching activities in Central and Eastern Europe.

P1707 The ESCMID Study Group for Antimicrobial Resistance Surveillance (ESGARS)

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Verona, I; Paris, F; Antwerp, B; Warsaw, PL; Linz, A; Smolensk, RUS; Madrid, E

The ESGARS has been established in 1997 by those participating in the WHO Meeting on 'The Present Status of Antimicrobial Resistance Surveillance in Europe'. Membership of ESGARS is open to those interested in resistance surveillance at local, national and international levels, including representatives from government or corporate bodies. Aims of the ESGARS are:

- To provide a uniting forum for those actively involved in antimicrobial resistance surveillance, in order to promote a better understanding of antimicrobial resistance.
- To provide opportunity to enhance co-operation and to establish links with and between networks of resistance surveillance programs.
- To promote awareness and facilitate the early detection of emerging antimicrobial resistance.
- To contribute to an understanding of the epidemiology of antimicrobial resistance in Europe.
- To improve access to European data on surveillance.
- To provide an opportunity for training in resistance detection and surveillance.

The Study Group is responsible for organizing ESCMID Post-graduate Education Courses in collaboration with the other ESCMID Study Groups whose interests overlap with those of the ESGARS:

- 9th Course 'The role of antimicrobial resistance surveillance for effective antibiotic policies', held in Verona (Italy) on December 8–9th, 1999 (co-organized with ESGAP and ESGNI)
- 18th Course 'Diagnostics, characterization and epidemiology of beta-lactamases', held in Orta San Giulio (Italy) on April 22nd-23rd, 2002 (co-organized with ESGNI).
- 26th Course 'The management of infections in adult ambulatory patients: from the laboratory to the clinic', to be held in Smolensk (Russia) on December, 11th–13rd, 2003.

The ESGARS is presently involved in producing the European Recommendations for Antimicrobial Resistance Surveillance, through a consensus process involving all members of the Study Group.

P1708 ESCMID Study Group on Epidemiological Markers (ESGEM)

K. Towner on behalf of the ESGEM Executive Committee
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Molecular typing is now an essential component of the microbiology and control of infection services provided across Europe and in many other countries of the world. Recent quantum advances in comparative genomics have yielded a wide choice of powerful methods for studying the genomic relatedness of microbes. The epidemiology of microorganisms does not recognize international boundaries. Collaboration between scientists working in different countries is therefore essential to standardize methodology and to track the spread of particular virulent or resistant microbial pathogens. ESGEM is a group of ESCMID members who share an interest in epidemiological typing systems. The key objectives of ESGEM are:

1. To critically evaluate microbiological typing systems and make recommendations (guidelines) for their appropriate use.
2. To promote collaborative research into microbiological typing systems and to develop standardized methodology for specific pathogens.
3. To provide opportunities for the exchange of ideas and the development of consensus strategies at ECCMID symposia and workshops. ESGEM will also continue to play a leading role in organizing the triennial International Meeting on Microbial Epidemiological Markers (IMMEM).
4. To organize multicenter studies and practical training workshops.
5. To work with individuals and companies active in this research area to foster the development of further technological advances in microbial typing.

P1709 ESCMID Study Group on Toxoplasmosis (ESGT)

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Stockholm, S

Toxoplasma gondii has a worldwide distribution causing a chronic infection in $>10^9$ people. Toxoplasmosis in immunocompromised patients is usually due to reactivation of latent infection. However, it may be transmitted by blood or blood products and by transplanted organs. In contrast to the majority of immunocompetent patients, immunocompromised ones may have serious sequelae. In the context of bone marrow transplantation and peripheral blood stem cell transplantation, profound immunosuppression leads to a high rate of infectious complications. Toxoplasmosis has been considered a rare opportunistic infection in patients who undergo BMT or organ transplantation. A reappraisal of the prevalence of toxoplasmosis in various groups of immunocompromised patients and its clinical impact is needed. Further, the recent developments in diagnostic assays, including PCR assays, need to be evaluated in large number of patients, preferably in a multicenter study, because the number of these patients is comparatively small in each single center. ESGT is a group of ESCMID members who share an interest in different aspects on the interaction between humans and *Toxoplasma gondii*. The primary focus initially will be on the:

1. Provide information of the prevalence of toxoplasmosis in immunocompromised patients in different parts of Europe
2. Provide information on clinical policies and practices within Europe
3. Provide information on the risk profile for toxoplasma infection in immunocompromised patients
4. Provide information on the diagnostic value of different tests
5. Generate a common European policy proposal for the diagnosis and management of toxoplasmosis in immunocompromised patients

P1710 European Study Group on Coxiella, Anaplasma, Rickettsia and Bartonella (ESCAR)

P. Brouqui
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ESCAR encourage basic and applied research in all aspects of rickettsiology and rickettsial diseases and foster exchange of information and/or materials among scientists engaged in the research on rickettsiae and rickettsial diseases through periodic meetings, and other devices or instruments as may be appropriate. ESCAR will encourage recruitment and training of young scientists in rickettsiology and rickettsial diseases. We consider and make recommendations on scientific and policy matters pertaining to rickettsiae and rickettsial diseases as may be desirable or necessary for the advancement of basic and applied knowledge in this field. We advise governmental and health agencies, when appropriate, on matters relevant to rickettsiae and rickettsial diseases. The field of research is epidemiology, genomics and taxonomy, diagnosis, physiopathology and immunology, and therapy of rickettsiosis, bartonellosis, anaplasmosis, and coxiellosis of human and animals. ESCAR is a small society of about 300 members made of medical doctors, scientists, microbiologists, veterinarians, pharmacists and others. We meet once a year at the ESCMID and we organize an international meeting every three years in Europe. We support a European network on diagnostic of tick-borne diseases. ESCAR members belong to most European countries and also from other foreign countries including USA, Japan, Russia and others.

P1711 ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria (ESGARAB)

C. E. Nord
Stockholm, S

Anaerobic bacteria are the predominant constituents of the normal microflora on the skin and the mucous membranes of the human body. They also act as potent pathogens in a variety of endogenous infections. All types of infections occurring in humans may involve anaerobic bacteria and no organ or tissue of the body is immune to infections with these microorganisms. There are four major sites of anaerobic infections: respiratory, intra-abdominal, female genital tract, skin and soft tissue infections. The bacteria most commonly involved in these infections are: *Bacteroides fragilis* group, *Prevotella* species, *Porphyromonas* species, *Fusobacteria*, *Peptostreptococci* and *Clostridia*. Five groups of antimicrobial agents are active against most anaerobic bacteria of clinical importance. These are nitroimidazoles such as metronidazole or tinidazole, carbapenems such as imipenem or meropenem, chloramphenicol or thiamphenicol, combinations of beta-lactam drugs together with a beta-lactamase inhibitor (clavulanate, sulbactam or tazobactam) and newer quinolones such as garenoxacin, moxifloxacin and sitafloxacin. Clinical usage of antimicrobial agents has been accompanied by the isolation of antimicrobial-resistant bacteria. During the last years there have been reports showing increasing numbers of anaerobic bacteria resistant to different antimicrobial agents. Resistance in anaerobic bacteria has a significant impact on the selection of antimicrobial agents for empirical therapy. The development of antibiotic resistance in anaerobic bacteria has been documented for beta-lactam drugs, clindamycin, macrolides, tetracyclines and nitroimidazoles. The *B. fragilis* group is more resistant to antimicrobial agents than most other anaerobic bacteria. The *Bacteroides* genus and new genera *Prevotella* and *Porphyromonas* have become increasingly resistant to many antianaerobic agents. *Fusobacterium* strains resistant to beta-lactam drugs are relatively frequent. Resistant *Clostridia* and *Propionibacterium acnes* have also been reported. The resistance mechanisms in anaerobic bacteria are: (i) hydrolysis of the antimicrobial drug by several enzymes before reaching the site of action (most common, sometimes plasmid mediated); (ii) decreased permeability of the organisms; (iii) modification at the site of action of the antimicrobial agent; (iv) efflux mechanisms which eliminate the antimicrobial drug from the bacterial cell. The aim of ESGARAB is (i) to investigate the antimicrobial resistance patterns in anaerobic bacteria in Europe (ii) to investigate the antimicrobial resistance mechanisms in anaerobic bacteria (iii) to develop standardization methods for testing antimicrobial agents against anaerobic bacteria (iv) to organize a symposium or workshop at each ECCMID, and (v) to organize scientific meetings and postgraduate education courses.

P1712 European Helicobacter Study Group

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Background: This group was founded in 1987 during a meeting in Copenhagen (Denmark). It was run informally for several years and became an official association under French law in 1997. A specific feature of this group is to be multidisciplinary, i.e. in addition to gastroenterologists, it includes pathologists, microbiologists, immunologists, internists, epidemiologists, pediatricians, etc.
Aim: The aims of the Association are to promote the research and to diffuse the medical, scientific and technical knowledge of the bacterium named *Helicobacter pylori*, its associated diseases and related microorganisms.
Activities:

- Organization of a yearly meeting since 1988. The annual 2 and a half day Workshop has been held in different countries of Western Europe and once in the US. It has gathered between 500 and 2700 participants. The 16th edition, currently in preparation, will be held in Stockholm, Sweden, during the first week of September 2003.
- Technical courses have also been organized in the past on laboratory methods, epidemiology applied to gastric cancer, and clinical trials.

- *H. pylori* sessions have been organized in the context of the UEGW yearly since 1992, and in the context the European Congress of Clinical Microbiology and Infectious Diseases every 2 years since 1989, yearly after 2000.
- Post-graduate courses have been organized for doctors from Eastern Europe (in 1992 in Prague) and for Russian doctors in 1998 and 2001 in collaboration with the Russian Society of Gastroenterology.
- Conferences to prepare 'Guidelines for the Management of *H. pylori* Infection' have been held twice in Maastricht and were published as the Maastricht Consensus Reports (Gut, APT).
- A yearly publication has been produced since 1994 which summarizes the news of the year in the field. It was published as a supplement of Current Opinion in Gastroenterology until 2001 and now as a supplement of Helicobacter.
- European multicenter studies concerning the evolution of gastritis (Eurohepygast) on resistance to antibiotics have been carried out.
- A website, created 5 years ago, proposes among others the abstracts of the meeting and a summary of the oral sessions. A section relative to treatment aid is also available.
- Fellowships have been attributed for studies in the field.
- A Pediatric Task Force has been created with specialists from EPSGHAN, which produced a position paper on management of *H. pylori* infection in children.

Organization: The council is comprised of a member from each of the countries of the European Community plus Switzerland. Most of them are representatives of national groups. The council meets 3 times a year, including one meeting at the Workshop. There is a President (1 year mandate) who is usually the organizer of the year's Workshop, a treasurer, and a secretary elected for 3 years. The running costs of the group are covered by part of the benefit from the Workshop, when available. The remaining surplus is used for fellowships. A number of projects are planned including a continuation of the yearly Workshop, the development of an important project on gastric cancer in the context of the 6th PCRD, and the publication of a text book.
Current officers:
President Prof T. Wadström, Lund, Sweden
Treasurer Prof A. Hirschl, Vienna, Austria
Secretary Prof F. Mégraud, Bordeaux, France

P1713 European Study on Antimicrobial Consumption (ESAC): a project funded by DG SANCO of the European Commission

H. Goossens on behalf of the ESAC Network

Summary: During a two year period, actions will be taken to harmonize the collection of antimicrobial consumption data in all EU countries. Retrospective data for ambulatory care and hospitals will be collected for the period 1997–2001. A prospective data collection system, based on a validated register of available antibiotic products linked to the correct ATC/DDD classification, will become effective in 2003. Standardized national data will be assembled in a European database for international comparison of antibiotic use in relation to antibiotic resistance patterns and socio-economic and general health parameters.
Problem: There is an established, albeit complex, relationship between consumption of antimicrobials and the prevalence of drug resistant bacteria. Although several antibiotic resistance surveillance programs are operational at the EU level, a program for the collection of data on the consumption of antimicrobials is lacking. In most European countries, some information on the consumption of antimicrobials at a national level exists. Large differences can be observed, however, in the structure and the accessibility of these databases. Moreover, in order to compare antimicrobial consumption at the EU level, internationally applicable methods need to be established including both a uniform classification system and a common unit of measurement.
Aim: This project aims to construct and maintain a European database on antimicrobial consumption based on standardized and validated national data using the DDD (Defined Daily Dose)/ATC (Anatomical Therapeutic Chemical) classification system. Quantitative consumption data will be complemented with a database offering an inventory of projects focused on antibiotic consumption (published research as well as ongoing projects). The database will be accessible for health authorities and scientists in order to link antibiotic consumption to resistance patterns and to assess the impact of intervention strategies at the community and hospital level.
Expected results: The main results of the pilot phase of the ESAC project will be the availability of an administrative database of antibiotic consumption in Europe. Retrospective data (1997–2001) from 27 countries are available. Data of one current EU country and two first wave EU countries are yet missing. In addition, data from Bulgaria, Croatia, Iceland, Norway and Turkey are available. Ambulatory care data are available from 25 countries and hospital care data from 22 countries. Retrospective data will be complemented with prospective data deriving from the continuous and comprehensive surveillance systems of antibiotic consumption, which should be operational in all European countries in 2003. A register of all antibiotic products in all package forms available in each country will be updated on a regular base and published on the ESAC website enabling the validation of the quality of the data delivery system. Quantitative consumption data will be complemented with a database offering an inventory of projects focused on antibiotic consumption (published research as well as ongoing projects). Potential applications: The results are targeted at the European Commission, health authorities and scientists in the different Member States. The database should offer a basis to monitor the use of antibiotics in a prospective and comprehensive manner. The results and recommendations should allow health authorities in the different Member States to promote evidence-based antibiotic use. The database should be used as an instrument to study and evaluate the link between resistance and antibiotic use, appropriate antibiotic prescribing, quality indicators of antibiotic consumption, ecological impact of antibiotic use, etc. The database will provide opportunities for further research and international collaboration.
Project web-site: http://www.esac.ua.ac.be.

P1714 European Antimicrobial Resistance Surveillance System (EARSS): a project funded by DG SANCO of the European Commission

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Summary: The European Antimicrobial Resistance Surveillance System (EARSS), funded by DG SANCO of the European Commission and coordinated by the Dutch National Institute for Public Health and the Environment (RIVM), is an international network of national surveillance systems, which collects comparable and validated antimicrobial susceptibility data for public health purposes. EARSS performs ongoing surveillance of antimicrobial susceptibility in *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis/faecium* causing invasive infections, and monitors variations of antimicrobial resistance in time and from place to place. In December 2002, around 600 microbiological laboratories serving some 970 hospitals in 27 countries provided susceptibility data on around 88 000 invasive isolates.
Problem: Antimicrobial resistance (AMR) is an emerging public health problem with local, national, and international dimensions as described in 'the Copenhagen Recommendations'. Antimicrobial resistance is clearly an emerging problem, however, the precise impact of this problem is less clear to the European and scientific community. Before being able to quantify the impact on public health it is necessary to have more comparable surveillance data available. One of the recommendations made at the EU Conference 'The Microbial Threat' in 1998 was that a European surveillance system of antimicrobial resistance should be set up, therefore EARSS has been funded.
Aim: EARSS aims to obtain comparable and reliable antimicrobial resistance data of main indicator pathogens in Europe to monitor antimicrobial resistance in time and from place to place. EARSS also aims to assess risk factors for antimicrobial resistance and EARSS aims to enable policy makers and health care workers to monitor the impact of their interventions.
Achieved results (ongoing project): For pathogens (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus fecalis/fecium*) causing invasive infections, resistance levels are available for important groups of antimicrobials from 27 European countries. In the EARSS annual report 2001, results are described in detail for all four pathogens collected in 2001, as well as trends in penicillin nonsusceptible *S. pneumoniae* (PNSP) and methicillin resistant *S. aureus* (MRSA) during the period 1999–2001. Aggregated information is directly available to health care workers, policy makers, and a wider public, at an interactive website (http://www.earss.rivm.nl). From EARSS data, it can be concluded that proportions of antimicrobial resistance vary markedly between European countries, most likely as a result of differences in hospital infection control and the consumption of antibiotics.
Potential applications: Policies to combat resistance should be tailored specific to country and hospital level. The results as presented recently in the EARSS annual report 2001 emphasize the need to implement the Council Recommendations on the Prudent Use of Antibiotics in Human Medicine. As laid down in the Council Recommendations, it has recently been decided

that multidisciplinary organizations, called Intersectorial Coordinating Mechanisms (ICMs), will be established at the national level. The ICMs will be responsible for information exchange and co-operation between the parties involved at the national level. The ICMs are responsible for implementing the Council's recommendations and should consider the recommendations as formulated in the EARSS Annual Report 2001. This applies particularly to countries with high proportions of resistance for all bacterial species. In order to get more insight into the causes and mechanisms of these striking differences, in the near future several research initiatives will be set up in close cooperation with the EARSS network. EARSS already cooperates closely with the project 'European Surveillance on Antimicrobial Consumption'(ESAC). EARSS and ESAC will start to cooperate with the project 'Self-medication of Antimicrobials and Resistance Levels in Europe' (SAR) before the end of 2002, and close cooperation with the project 'Antibiotic Resistance in Mediterranean countries' (ARMed) will start in 2003. Project web-site: aggregated information is directly available to health care workers, policy makers, and a wider public, at an interactive website (http://www.earss.rivm.nl).

P1715 Antibiotic Resistance – Prevention and Control (ARPAC)

F. MacKenzie on behalf of the ARPAC Steering Group

ARPAC is a concerted action project funded by the Research Directorate-General of the E.C. ARPAC stands for Antibiotic Resistance, Prevention and Control. Its objectives are to lay the foundations for a better understanding of the emergence and epidemiology of antibiotic resistance and to evaluate and harmonize strategies for prevention and control of antibiotic resistant pathogens in European hospitals. Specific aims are to identify antibiotic polices and prescription patterns associated with low resistance rates and to identify infection control policies associated with lower incidence rates of transmissible antibiotic resistant strains. The study is being conducted by four ESCMID study groups, ESGAP, ESGARS, ESGNI and ESGEM. The project website is at http://www.abdn.ac.uk/arpac/index. Data will be collected from all willing European hospitals wishing to join the project. Data will include: antibiotic consumption, antibiotic resistance rates, DNA typing methods, information on antibiotic prescribing policies and information on antibiotic stewardship practices and infection control policies and practices. The data will be modeled to identify (i) antibiotic policies associated with lower resistance rates and (ii) infection control policies associated with low incidence rates of transmissible antibiotic resistant bacteria. Specific strategies leading to low rates of antibiotic resistance will be recommended and will form the basis for future intervention studies. The ESCMID Study Group on Antibiotic Resistance Surveillance (ESGARS) will compile an inventory of antibiotic resistance data and development of a strategy for collection, collation, critical assessment and dissemination of resistance data. The ESCMID Study Group on Antibiotic Policies (ESGAP) will compile an inventory of antibiotic consumption, prescribing habits, policies and stewardship in European hospitals. The ESCMID Study Group on Nosocomial Infections (ESGNI) will compile an inventory of European hospital policies and practices for controlling transmissible antibiotic resistant microorganisms. The ESCMID Study Group on Epidemiological Markers (ESGEM) will develop a DNA typing database and data exchange format for tracking epidemic antibiotic-resistant microorganisms. The project will culminate with a consensus conference to discuss results, which will be held in the autumn of 2004.

P1716 EUCAST – the European Committee on Antimicrobial Susceptibility Testing

G. Kahlmeter, D. Brown, A. MacGowan, F. Goldstein, J. W. Mouton, A. C. Rodloff, M. Steinbakk, M. Kalin, P. Urbaskova, A. Vatopoulos, the EUCAST General Committee

The EUCAST, formed in 1996 and restructured at ECCMID in Milan 2002, is the European Committee on Antimicrobial Susceptibility Testing. It consists of a General Committee with representatives from all European countries, from the pharmaceutical industry, and from in vitro media and device industries. EUCAST is led by a Steering Committee appointed by ESCMID and consisting of a Chairman, a Scientific Secretary, six National Breakpoint Committee representatives and two representatives of the EUCAST General Committee. Decisions are made by the Steering Committee after consultation with the General Committee.

Since being re-structured in 2002, EUCAST has:

- Agreed on a principal model for harmonizing breakpoints for new antibiotics in Europe.
- Proposed and is testing (with fluoroquinolones) a model for appraising and hopefully harmonizing breakpoints of registered antibiotics – see EUCAST symposium at ECCMID, Glasgow 2003.
- Devised web-based software for the collection and presentation of wild-type MIC distributions of relevant drug/bug combinations. A link will be placed shortly from the EUCAST website – http://www.eucast.org
- Agreed to define wild type cut-off values for the measurement of antimicrobial resistance development.
- Worked closely together with the EARSS (the European Antimicrobial Resistance Surveillance System)
- Opened discussions with EMEA, NCCLS, EFPIA, STMA, NEQAS and other organizations with interests and activities in the field of antibiotics.

EUCAST is an open organization that invites anyone with an interest in antimicrobial agents in general and antmicrobial breakpoints in particular to make contact, either through the ESCMID or directly with the Chairman (gunnar.kahlmeter@ltkronoberg.se) or the Scientific Secretary (dfjb2@cam.ac.uk) or through one of the National Breakpoint Committees.